[COMMITTEE PRINT]

RECENT ADVANCES IN THE PLANT SCIENCES: APPLICATIONS TO AGRICULTURE AND AGRICULTURAL PRODUCTS

REPORT

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FOR THE

SUBCOMMITTEE ON
INVESTIGATIONS AND OVERSIGHT

TRANSMITTED TO THE

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SCIENCE AND TECHNOLOGY
U.S. HOUSE OF REPRESENTATIVES
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June 28, 1984

LETTER OF TRANSMITTAL

Honorable Don Fuqua, Chairman
Committee on Science and Technology
U.S. House of Representatives
Washington, D.C. 20515

Dear Mr. Chairman:

I am pleased to transmit to you a report prepared by the Congressional Research Service for the Subcommittee on Investigations and Oversight. The report, entitled, "Recent Advances in the Plant Sciences: Applications to Agriculture and Agricultural Products," describes the applications of advances in biotechnology to agriculture.

Recombinant DNA technology and other genetic engineering techniques will have a dramatic impact on all segments of society. Nowhere will this impact be greater than in agriculture, where the applications of biotechnology promise to produce new varieties of crops, crops better able to grow in arid areas, disease resistance crops, and crops able to manufacture their own fertilizer. While many of these benefits are probably decades away, substantial progress is now being made, and there is reason to believe that there will be significant results in the near term.

Because of this potentially enormous impact, the Subcommittee asked the Congressional Research Service to develop a primer about these exciting scientific advances which would provide useful information to a wide audience in the agricultural community. This primer, devoted to plant sciences, is a companion to the report prepared for the Subcommittee last year by the Congressional Research Service. That report reviews the applications of biotechnology to livestock production and is included as an appendix to this report. Both primers draw heavily on the Subcommittee's hearings on the applications of biotechnology. The primers do not address policy questions, such as manpower needs in research or research priorities, but rather these primers are intended as a scientific status report.

I would like to express my appreciation to James McCullough, Chief of the Science Policy Research Division, and Judith Johnson, Analyst in Life Sciences, of the Congressional Research Service for their excellent work in preparing this report and for their continuing support to the Subcommittee in its exploration of biotechnology issues affecting society.

Sincerely,

Albert Gore, Jr.

Albert Gore, Jr., Chairman
Subcommittee on Investigations
and Oversight

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LETTER OF SUBMITTAL

June 22, 1984

Honorable Albert Gore, Jr.
Chairman, Subcommittee on
Investigations and Oversight
Committee on Science and Technology
House of Representatives
Washington, D.C. 20515

Dear Mr. Chairman:

It is my pleasure to submit the report entitled Recent Advances in the Plant Sciences: Applications to Agriculture and Agricultural Products, prepared at the request of the Subcommittee on Investigations and Oversight of the House Committee on Science and Technology.

This document discusses recent advances in plant genetic engineering and their potential applications in agriculture. It complements a prior report on similar advances in the animal sciences. Both reports were prepared by Judith A. Johnson, Analyst in Life Sciences, of our Science Policy Research Division.

On behalf of the Congressional Research Service, I thank you for the opportunity to assist your subcommittee in the preparation of this report.

Sincerely,

Gilbert Gude
Director

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RECENT ADVANCES IN THE PLANT SCIENCES: APPLICATIONS TO AGRICULTURE AND AGRICULTURAL PRODUCTS

Judith A. Johnson
Analyst in Life Sciences
Science Policy Research Division
April 16, 1984

INTRODUCTION

Advances in plant tissue culture and gene manipulation are beginning to revolutionize plant breeding. The application of these techniques in plant breeding is expected to add $5.6 billion to the annual value of crops before the year 2000. 1/ The value of seed incorporating improvements from the use of these techniques is also expected to increase dramatically, from about $8 million in 1985 to $6.8 billion by the year 2000. 2/

Genetic engineering and plant tissue culture will enable researchers to cope with the many challenging problems agriculture faces today and in the near future. Oil prices have pushed the cost of essential petroleum based fertilizers out of reach of all but the wealthiest countries. Water is in short supply and will become even more scarce in the years to come. Salt accumulation in irrigated soils is another serious problem which is expected to become worse with time. Pest and plant diseases are becoming resistant to previously developed pesticides. And, three million acres of the 400 million acres of U.S. farm land are lost to erosion and urban development each year.

As specified by the subcommittee, this report will discuss the applications of recombinant DNA and tissue culture techniques in the plant sciences as they relate to agriculture. Section I describes the relevant techniques and

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1/ The New Plant Genetics. Report by L. Williams Teweles and Company, Milwaukee, Wis. Dec. 1983. 700 p.

2/ Ibid.

section II their potential use in plant breeding. A second report entitled "Animal Sciences: Advances in Reproductive and Health Technologies," also prepared by the Congressional Research Service for the Subcommittee on Investigations and Oversight is included as an appendix to provide complementary information on the agricultural applications of genetic engineering.

I. TECHNIQUES

A. TRADITIONAL OR CONVENTIONAL PLANT BREEDING

"Agriculture is both the oldest and the largest of the world's industries." 3/ In fact, the plant breeding techniques used by the first farmers 10,000 to 12,000 years ago were still in use, with few refinements, until the turn of the century, just 80 years ago. Until the 1900s, crop improvement was in the hands of farmers who selected seed from preferred plants for subsequent sowing. The rediscovery of Mendel's "laws of inheritance" in 1901 (originally reported in 1865) resulted in a tremendous scientific interest in genetics, especially in plants since Mendel had determined his principles by working with peas. Mendel's laws introduced the idea that through selective breeding, man could recombine traits (i.e., genes) from natural sources into combinations that were desired for agricultural production but could not be found in nature. The concept of selective breeding is part of what is now referred to as traditional or conventional plant breeding.

The conventional breeder can and has made extraordinarily large changes in plants with remarkable precision. In combination with the use of pesticides, herbicides and fertilizers, the genetic improvement of cultivated plants has resulted in spectacular increases in yield. For example, "between 1940 and 1980 the combined production of 17 major crops in the United States increased

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3/ Barton, Kenneth A., and Winston J. Brill. Prospects in Plant Genetic Engineering. Science, v. 219, Feb. 11, 1983. p. 671.

242 percent. . . . This large increase in production was obtained with an increase in the area of cultivated land of only 3 percent." 4/

However, conventional breeding does have some disadvantages. With few exceptions, the conventional breeder can manipulate the genes and chromosomes of plants only within the confines of nature, that is, within the limits of sexual reproduction. For this reason, the production of new plant varieties is often slow, and combinations of unrelated plants (such as corn and soybeans) or even related types that fail to combine sexually (like some strains of rice) is impossible. "The time required for development of new plant varieties using conventional approaches has to some extent restricted corporate interest in plant breeding. This is because corporate research programs generally adhere to time frames of 3-5 years." 5/ For example, "some outstanding crops, such as winter barley, produce only one generation a year; with the best will in the world, and starting from good parent material, it takes 12 years to produce a genuinely new variety. At that rate, we can expect only two complete 'generations' of varieties of barley by the end of the century." 6/ The techniques of tissue culture and genetic engineering are currently being used to circumvent some of the obstacles of traditional breeding.

4/ Borlaug, Norman E. Contributions of Conventional Plant Breeding to Food Production. Science, v. 219, Feb. 11, 1983. p. 691.

5/ Sharp, William R., David A. Evans, and Phillip V. Ammirato. Plant Genetic Engineering: Designing Crops to Meet Food Industry Specifications. Food Technology, v. 38, Feb 1984. p. 112.

6/ Tudge, Colin. The Future of Crops. New Scientist, May 26, 1983. p. 548.

B. PLANT CULTURE TECHNIQUES

1. Vegetative or Clonal Propagation

A number of plants can be asexually reproduced by removing parts of their structures and growing them into completely new plants. This technique, called vegetative or clonal propagation, is very old and probably began over 8,000 years ago with multiplication of select wine grape cuttings. 7/ It continues to be of great importance in horticulture since it ensures the reproduction of plants with desired traits. When practiced on a large scale vegetative propagation provides a high degree of uniformity among plants and their products.

However, the conventional methods of vegetative propagation do have several disadvantages. Relatively large macroscopic pieces of the parent plant are used, which often harbor diseases and pests. Also, for many important species, the use of large plant pieces result in slow multiplication rates. Some species cannot be propagated at all by current conventional propagation methods. For others, only immature plants, whose agronomic characteristics have not yet been completely evaluated, respond to conventional propagation techniques. Because of these problems, methods of using smaller even microscopic pieces of the parent plant tissue have been developed over the past 20 years. Tissue culture and micropropagation have become increasingly important commercial methods of asexual reproduction in many plant species.

7/ Constantin, M. J. Historical Perspectives and Introduction. In: Propagation of Higher Plants Through Tissue Culture. National Technical Information Service, U.S. Dept. of Commerce, 1979. p. 11-13.

2. Plant Cell and Tissue Culture/Micropropagation

The first documented effort to grow plant tissue in culture occured in 1902 when a German plant physiologist, Gottlieb Haberlandt, reported his unsuccessful attempts at regenerating plants from single cells. Very little progress was made in plant culture methods until 1939 when tobacco and carrot tissues were successfully grown in culture. In the late 1950s, the regeneration of plants from tissue culture was achieved. The first commercial application of micropropagation was based on the discovery in the 1960s that Cymbidium orchids could be efficiently multiplied by meristem tip culture. Meristems are located at the growing points of plants, areas of rapidly dividing cells at the tip of a stem, root or branch. By using tissue culture methods, many thousands of orchid plants can be produced over a period of one to two years. Conventional methods of clonal propagation could only produce a few orchids in the same period of time. Orchid growers quickly accepted micropropagation as a means of rapidly multiplying their superior plants. "Because of the high value of orchids as potted plants and cut flowers, the high cost of micropropagation was commercially justified." 8/

In the 1970s, micropropagation techniques were extended to a wide range of plant types. It is necessary to develop standardized methods for each new species cultured, and frequently for varietal or cultivar differences within a species as well. Each method is determined by trial and error, altering various growth media components including vitamins, amino acids, inorganic salts, and most importantly hormones, until the optimal mix is acheived. All such cultures are grown in facilities with controlled temperature and light.

8/ Aynsley, John, Donald Diotz, and George M. Kidd. Micropropagation Enters a New Commericalization Phase. Bio/Technology, Apr. 1983. p. 167.

A major problem in developing micropropagation techniques for wide spread application has been the regeneration of complete plants from single cells, especially in such important groups as the grasses, cereals, and woody species. The transformation from single cells, to groups of cells, to tiny plantlets with shoots and roots is achieved by varying growth media components, particularly the plant hormones. Often unique variants are found among plants regenerated from single cells. While this occurance was initially regarded as a disadvantage of the technique, it is currently being used on an experimental basis by plant breeders in several species as a source of generating genetic variability. This will be discussed at greater length in the following section.

A significant advantage of tissue culture is that traits or characteristics cells can be evaluated and screened more efficiently using cells in the laboratory rather than whole plants, thus decreasing the cost and space needed for growing the plants in the field. "As many as 5 million to 500 million cells or cell aggregates may be contained in a single culture flask. Assuming that single cells can be selected and ultimately regenerated into whole plants, the efficiency in sheer number of experimental units is striking, especially when contrasted to whole plants numbering only in the thousands per acre of land." 9/ Tissue culture allows the plant breeder to increase the supply of limited plant material and to reduce the time required to introduce selected genetic traits.

At the present time, micropropagation "is credited with the production of over 20 million plants annually in the United States and Europe, and of 60 million worldwide. Orchids now represent only a small fraction of this production." 10/

9/ Collins, G. B. Plant and Cell Tissue Culture: An Overview. In: Priorities in Biotechnology Research for International Development. National Academy Press, Wash., D.C., 1982. p. 231.

10/ Aynsley, Diotz and Kidd, Micropropagation Enters a New Commercialization Phase, p. 167.

Other examples of tissue culture propagated plants include cassava, peach, asparagus, strawberry, cauliflower, Boston fern, various lilies, and the date, coconut and oil palms. 11/

3. Somaclonal Variation

As mentioned briefly in the previous section, genetic variants can be generated during plant cell culture, particularly when tissue other than meristems (e.g., leaves, stems, and tubers) are used. Variations can include traits such as plant size, flower morphology, and pigmentation. While some traits are obviously detrimental, such as infertility or growth reduction, others may be beneficial. Since such variants arise from somatic cells (vegetative or non-sex cells) this capacity or property is called somaclonal variation. "Many important varieties of clonally propagated crop plants have arisen from such vegative mutations. Familar examples are the pink grapefruit, the navel orange, the nectarine, and several varieties of potato." 12/ Genetic variability also occurs in plants regenerated from tissue cultured pollen or male gametes and is called gametoclonal variation.

Genetic variation resulting from tissue culture has been demonstrated in tomato, potato, sugarcane, wheat, corn, rape, tobacco, oat, and rice. The mechanism or cause of such variation is unknown, but may be due to chromosomal rearrangements or gene mutations, possibly caused by the culture medium.

Since genetic mutations are relatively rare and frequently recessive events, traditional plant breeding methods are often inefficient at mutant recovery.

11/ Teutonico, Rita and Dietrich Knorr. Plant Tissue Culture: Food Applications and the Potential Reduction of Nutritional Stress Factors. Food Technology, Feb. 1984. p. 122.

12/ Shepard, James F. The Regeneration of Potato Plants from Leaf-Cell Protoplasts. Scientific American, May 1982. p. 154.

Tissue culture allows large populations of genetic variants to be screened at one time. It also makes possible the development of selection schemes, similiar to those used on bacteria, that would be impossible in intact plants. Such selection schemes could also be used on variants derived from the exposure of cultured plant cells to mutagenic agents as well as on somaclonal variants occuring spontaneously in tissue culture. The time required for developing new disease resistant varieties, for example, could conceivably be reduced by employing these methods.

For such a system to be useful in plant breeding, however, two criteria must be met. First, the variant cell must be capable of regeneration into a complete plant. Second, the variant must have a true genetic change which is expressed at the whole plant level and is stably transmitted to successive sexually produced generations. However, plant tissue culture only allows for the selection of traits expressed at the cellular level, a level of complexity distinct from that at which the trait's expression is ultimately desired. "Not all traits expressed by the whole plant are expressed by the cultured cell," and vice versa. 13/ For example, the selection of plants with altered time of flowering would be difficult at the tissue culture level since such a trait is due to complex interactions between highly differentiated cells, tissues and organs present only in the whole plant. It is hoped that as the technique of plant cell culture is refined and extended to more species, its corresponding range of applications in plant breeding will be increased.

13/ Chaleff, R. S. Isolation of Agronomically Useful Mutants from Plant Cell Cultures. Science, v. 219, Feb. 11, 1983. p. 679.

4. Protoplast Fusion

Protoplasts are living cells, plant or bacterial, which have had their cell walls enzymatically removed. Efficient techniques for plant protoplast isolation were developed in the late 1960s, and the polyethylene glycol method of protoplast fusion, introduced in 1974, resulted in high frequency recovery of fused protoplasts. Initially, efforts were focused on protoplast fusions between distantly related species "either to produce combinations not possible via conventional breeding or to make combinations that could be easily distinguished from the parental species." 14/ However, combinations between, for example, soybean and tobacco or carrot and petunia, would not allow the regeneration of hybrid plants from the fused cell lines. Although plants have been regenerated from fusions combining more closely related species, such as tomato and potato, most of these hybrids are sexually sterile, thus preventing their incorporation into breeding programs. Emphasis is currently on the the recovery of fertile hybrids, which have either unique combinations of cytoplasmic characteristics or only a few transferred genes. 15/

Not all of an organism's genetic information is stored solely in the cell nucleus. Small intracellular bodies called organelles (analagous to organs) which perform specific cell functions also have their own DNA: in plants, chloroplasts and in plants and animals, mitochondria. The chloroplast is the site of photosynthesis in plant cells; the mitochondrion produces energy via

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14/ Evans, David A. Agricultural Applications of Plant Protoplast Fusion. Bio/Technology, May 1983. p. 254.

15/ Ibid.

respiration in both plant and animal cells. The creation of cytoplasmic hybrids is an attempt to focus on the transfer of organelle-encoded traits. "During fertilization, the male cytoplasm is preferentially excluded from the developing embryo. Thus, in most crops, conventional sexual hybrids contain and express only maternally derived cytoplasmic genes. Since protoplast fusion combines both parent cytoplasms into a single protoplast, fusion can be used to produce unique nuclear-cytoplasmic combinations not possible using conventional breeding." 16/ Several important traits are thought to be contained in cytoplasmic DNA, such as plant male sterility in mitochondrial DNA and herbicide resistance in chloroplast DNA. Cytoplasmic hybrids are formed by combining protoplasts of one species which have had their nuclei inactivated or removed and protoplasts of a second species which have intact nuclei.

Most of the hybrids produced by protoplast fusion have been of the Solanaceae family, examples of which are the potato and tomato. The difficulty in other species seems to be the regeneration of whole plants from protoplasts. Very little success has been reported in plant regeneration for such important crops as cereals and legumes. At the present time, protoplast fusion provides "little control over what genetic information is retained and what is eliminated, and [it] lacks the potential precision of recombinant DNA methods. However, until the process of directed transformation with cloned genes reaches a higher level of sophistication, protoplast fusion offers a means for introducing genes from unconventional sources." 17/

16/ Sharp, William R., David A. Evans, and Philip V. Ammirato. Plant Genetic Engineering: Designing Crops to Meet Food Industry Specifications. Food Technology, Feb. 1984. p. 116.

17/ Shepard, James F., Dennis Bidney, Tina Barsby, and Roger Kemble. Genetic Transfer Through Interspecific Protoplast Fusion. Science, Feb. 11, 1983. p. 687.

C. GENETIC ENGINEERING IN PLANTS

1. Methods in Dicots: Ti Plasmid

The simplest and most successful method for the transfer of genes into plants has been the natural system found in the common soil bacterium Agrobacterium tumefacien.

An infection of this microorganism causes plant cells to proliferate and form a gall or tumor in a wide range of dicotyledonous plants or dicots*. Such tumors occur at wound sites often near the junctions of the root and stem, called the crown. Crown gall disease "causes considerable loss in certain crops, notably grapes, stone fruits and ornamental plants. Since the turn of the century, it has been studied by plant pathologists because of its economic impact." 18/

A. tumefaciens was identified as the causative agent in crown gall disease in 1907 by researchers at the U.S. Department of Agriculture. In the late 1940s, scientists at the Rockefeller Institute found that unlike normal plant cells or tissue, crown gall cells grow rapidly in tissue culture without the addition of any plant hormones, even when free of A. tumefaciens. It seemed as though the bacteria in its initial infection process had somehow permanently changed or transformed the plant cells into tumor cells. In the 1960s, researchers in France "found that crown gall cells synthesize a class of novel

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18/ Chilton, Mary-Dell. A Vector for Introducing New Genes into Plants. Scientific American, June 1983. p. 51.

* Dicots are one of the two major divisions of flowering plants, the other being monocotyledons or monocots. Dicots are characterized by having broad leaves with net veins; monocots by long, parallel veined leaves. Examples of dicots are all flowering trees and shrubs such as oak, elm and walnut, as well as most fruits and vegetables: bean, potato, lettuce, pepper, apple, and melon. Examples of monocots are palms, bamboo, pineapple, banana, the cereals such as corn, wheat, oat, rye, rice, barley and all the grasses.

compounds (which they named opines) that are not present in normal cells of the same plant." 19/ Two such opines are octopine and nopaline. Each strain of infectious A. tumefaciens can cause plant tumor cells to synthesize one type of opine. The infecting bacteria then use that particular opine (and only that opine) as a source of food.

The fact that these properties could be transferred to even distantly related species of noninfectious Agrobacterium indicated that the tumor induction and opine synthesis information were on some form of mobile genetic element, possibly a virus or a plasmid. A plasmid is a small circle of DNA, distinct from the bacterial chromosome, which usually carries supplementary genes "such as those affecting pathogenicity, the metabolism of rare compounds or resistance to antibiotics." 20/ In 1974, scientists at the University of Ghent, Belgium, found very large plasmids in all the infectious strains of Agrobacterium but not in the noninfectious strains. The plasmid was named Ti, for its tumor inducing capability. Researchers at the University of Washington in Seattle subsequently showed in 1977 that the Ti plasmid is able to transfer a segment of its DNA (called T-DNA) into the nuclear DNA of infected plant cells. The remaining portion of the plasmid enables the bacteria to utilize the opines as a food source.

The Ti plasmid of Agrobacterium is a natural genetic engineering device. It allows the bacteria "to subvert the plant cell's metabolic machinery to make a substance that is of no use to the plant on which the bacteria (and hence also the symbiotic plasmid) can proliferate." 21/ Scientists at a number of

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19/ Ibid.

20/ Ibid.

21/ Ibid., p. 53.

research institutes and corporations are currently trying to adapt the Ti plasmid to transfer agriculturally important traits into crop plants.

Several of the initial obstacles to using the Ti plasmid as a genetic engineering tool have already been overcome. One problem was the expression of the transferred gene in the plant cell. Transfer alone was not sufficient for the new gene to function. The appropriate regulatory sequences,* recognized by the plant's internal "machinery," must be present in order for the gene to operate. The first foreign genes inserted into plants were from bacteria and failed to be expressed. Presumably this occurred because the bacterial regulatory sequences associated with the gene were not recognized by the plant. Later attempts with yeast and mammalian genes also failed, probably for the same reason. Researchers have circumvented this problem by using regulatory sequences that are already known to be recognized by the plant. One such sequence is the promotor adjacent to the Ti plasmid gene coding for synthesis of the opine nopaline. In January 1983, scientists at Monsanto, Washington University, and the University of Ghent announced the successful expression of bacterial genes in cultured plant cells. 22/ All three placed the foreign gene next to the nopaline promoter, thus forming what has been called a chimeric gene. In November 1983, scientists at the University of Wisconsin and Agrigenetics, a Colorado based seed company, reported the first successful expression of a bean storage protein gene in tobacco plants. 23/

22/ Herrera-Estrella, L., A. Depicker, M. Van Montagu, and J. Schell. Expression of Chimeric Genes Transferred into Plant Cells Using A Ti Plasmid-Derived Vector. Nature, v. 303, May 19, 1983. p. 209.

23/ Murai, N. et. al. Phaseolin Gene from Bean is Expressed After Transfer to Sunflower via Tumor-Inducing Plasmid Vectors. Science, Nov. 4, 1983. p. 476.

* Regulatory or promoter regions control the expression of adjacent genes, somewhat analogous to an on/off switch.

A second problem was the regeneration of healthy plants containing the functioning foreign gene. In February 1984, scientists at Monsanto reported the successful regeneration of plants containing an active bacterial gene. The bacterial gene for resistance to the antibiotic kanamycin was stably inherited through seeds in typical Mendelian fashion, and was expressed by the recombinant plants' offspring. 24/ The Monsanto scientists achieved regeneration by allowing the bacterial gene to be transferred via the Ti plasmid, but without the plasmid's tumor-producing genes. Scientists at the University of Ghent have also reported the regeneration of plants containing a functional foreign gene using methods similar to those of the Monsanto group. 25/ However, the inheritance of the new trait through seed was not reported.

A third problem is the timing of the foreign gene's expression in the new environment of the plant cell. Work in this area is crucial if recombinant DNA is to become an important tool in plant breeding. For example, breeders would be interested in improving the seed storage proteins in crops used for animal feed. Many do not contain a proper balance of nutrients (amino acids) to achieve maximum animal growth. Provided that the appropriate gene for transfer was found, it would be most useful for the gene to be expressed only within the seed, during its development and maturation. Expression elsewhere in the plant would be an unnecessary waste of both the plant's energy and of the protein itself. This specialized expression requires the identification and isolation of special regulatory or promotor regions.

24/ Horsch, R. B., R. T. Fraley, S. G. Rogers, P. R. Sanders, A. Lloyd and N. Hoffman. Inheritance of Functional Foreign Genes in Plants. Science, v. 223, Feb. 3, 1984. p. 496.

25/ Caplan, A., L. Herrera-Estrella, D. Inze, E. Van Haute, M. Van Montagu, J. Schell and P. Zambryski. Introduction of Genetic Material into Plant Cells. Science, v. 222, Nov. 18, 1983. p. 819.

Scientists are just beginning to perform such experiments with specialized promotors. The group at the University of Ghent have succeeded in linking a light sensitive promoter from the pea plant with a bacterial antibiotic resistance gene. This chimeric gene was introduced into tobacco cells using the Ti plasmid, and the cells were regenerated into tobacco seedlings. The transferred gene was able to switch on in lighted conditions, and off in the dark. 26/ 27/ The European research team is also working on using promoters found in the potato plant which only function in the tuber itself and not in other parts of the plant. 28/

A fourth and perhaps most challenging problem is the general lack of knowledge of genes that are likely to improve crop plants. The molecular basis of most desirable genetic traits in plants is unknown, and is often determined by many widely separated genes. "Within the entire kingdom of plants with its hundreds of thousands of species, perhaps only 20 genes have been characterized, and they are not all from the same plant, or even from a commercially significant species of plant. Yet each individual plant may contain about 30,000 different genes." 29/ Deciding which genes code for important agricultural traits such as larger yields is a very difficult problem. "The trouble is that there is no known way to find genes controlling such traits, most of which are probably governed by a number of genes and perhaps by elaborate regulatory mechanisms. Even if the appropriate genes could be identified, there is no

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26/ Ibid.

27/ Schmeck, Harold M., Jr. Artificial Gene Can Be Turn On or Off. The New York Times, Oct. 4, 1983. p. C3.

28/ Ibid.

29/ Tudge, The Future of Crops, p. 549.

known technique for isolating them by recombinant-DNA methods; only genes whose protein product is known have so far been isolated." 30/

Lastly, all the experiments performed to date using the Ti plasmid and Agrobacterium have used a very limited number of dicotyledonous plants, mostly tobacco and petunia. This will remain the case until regeneration method are developed for other agriculturally important species.

2. Methods in Monocots: Transposable Elements

As mentioned in the previous section, monocots are one of the two major divisions of flowering plants, and are characterized by, among other things, parallel veined leaves. While Agrobacterium and its Ti plasmid are an ideal genetic engineering system for dicots, such a system does not exist for monocots. At the present time, the most promising method of gene transfer for monocots, or at least for corn, is the transposable element system discovered by Barbara McClintock of Cold Spring Harbor Laboratory more than 30 years ago.

Dr. McClintock received the 1983 Nobel Prize in Medicine for her discovery in the 1940s of "mobile genetic elements." Today, these elements are understood to be segments of DNA that have the ability to move around the genome and control or affect many different genes. They have also been referred to as "controlling elements" (McClintock's term), "jumping genes" and "transposable elements."

Dr. McClintock's work was far ahead of its time and was not readily accepted by the scientific community of the 1950s and 1960s since her ideas were complex and contradicted the prevailing doctrine. She first presented her transposable element theory in 1951 at a symposium in Cold Spring Harbor, New York. Her presentation was based on six years of work studying the variegation of kernal

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30/ Chilton, A Vector for Introducing New Genes Into Plants, p. 59.

pigmentation in corn. The inheritance of coloration in corn kernals was known to be genetically unstable, that is, the patterns of corn kernal pigmentation could not be reliably predicted using the principles of genetics known at the time. Dr. McClintock concluded that two types of movable controlling elements were responsible for the observed color patterns. She named them Ds (for dissociator) and Ac (for activator).

Dr. McClintock hypothesized that both elements can move around the corn genome: Ac can move on its own; Ds requires the presence of Ac somewhere in the genome in order to move. In the absence of Ac, Ds elements are stable and their position can be determined by standard genetic mapping techniques. If Ds or Ac moves close to or into a chromosomal gene, however, the element can effect or terminate that gene's function or expression. Removal of the element sometimes results in normal expression of the affected gene. Movement of the elements can also result in chromosome breakage. Dr. McClintock followed the movement of Ac and Ds indirectly by the effects they had on kernal pigmentation and chromosome integrity. Recently, scientists at several research centers including the Cold Spring Harbor Laboratory, and the Carnegie Institute of Washington, have been able to confirm at the molecular level Dr. McClintock's findings on corn transposable elements.

Since these elements (and others discovered later) are capable of inserting into many new locations in corn DNA, they may be useful for inserting foreign genes into corn chromosomes. Work on this potential gene transfer system is currently underway in Australia, Germany, and the United States. Researchers in both Australia and Germany have isolated the corn Ds element. 31/ The Australian

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31/ Marx, Jean L. The Transposable Element of Maize Emerges. Science, v. 219, Feb. 18, 1983. p. 829.

group eventually plans to transfer Ds along with a gene for the enzyme alcohol dehydrogenase (ADH) into corn cells which lack ADH. 32/ Such cells cannot grow in the absence of oxygen. If the experiment succeeds, the cells will be able to grow in the absence of oxygen, thus indicating that Ds and the gene for ADH were indeed transferred into the plant cells. The Ac element has also been isolated by the U.S. group at the Carnegie Institute of Washington. 33/ Ac could possibly be used as described for Ds above, or as a tool for controlling the insertion of Ds.

It will be necessary to use corn cell protoplasts in order to allow Ds and its foreign gene to enter a plant cell. However, it has been very difficult to regenerate whole corn plants from protoplasts. Scientists at both Sungene Technologies Corporation and the University of Minnesota have had some recent success in corn plant regeneration. 34/ 35/

Most scientists do not believe that the transposable elements found in corn will have broad applications to other cereals. However, the cereals may possess their own undiscovered elements with functions similar to those found in corn.

3. Other Methods

Plant viruses are also being studied as potential gene transfer systems. Viruses are very small packages of DNA or RNA encapsulated in protein. As with the Ti plasmid, many viruses can insert genetic information into their hosts.

32/ Yanchinski, Stephanie. Maize Cells Tricked Into Accepting Foreign Genes. New Scientist, Sept. 8, 1983. p. 686.

33/ Ibid.

34/ Marx, A Transposable Element of Maize Emerges, p. 830.

35/ Sungene Field-Tests Tissue-Cultured Corn Plants, Seeks Varieties Faster. Biotechnology Newswatch, Jan. 16, 1984. p. 6.

By removing the virus' disease-producing capabilities and inserting the desired foreign gene, viruses could possibly be used as a plant gene transfer system. An advantage to a viral transfer system is that all plants are susceptible to viruses. Most work of this nature has been performed on the cauliflower mosaic virus (CaMV). A major drawback, however, is the size of the foreign DNA which CaMV can accept. To date, only pieces smaller than a gene have been stably inserted into CaMV DNA. In addition, CaMV has a limited natural host range and is not transmissible by seed. However, it can infect the whole adult plant and then move systematically throughout the entire host. Use of CaMV would therefore eliminate the need for intermediate steps involving tissue culture.

Gemini viruses also hold potential as a gene transfer system in plants since they infect a wide variety of species including legumes and cereals. They also have more room to accommodate foreign DNA. Scientists at the John Innes Institute in Great Britain have inserted foreign genes into the DNA of this virus and have succeeded in infecting corn cells with the recombinant virus. 36/ However, knowledge of the basic biology of this virus is rudimentary and is still being investigated. Cryptic viruses are also generating interest as a possible transfer system. They can be transmitted through seed, and they show virtually no disease symptoms (hence the name cryptic). A major drawback to the use of these viruses is that they are composed of RNA, not DNA. The methods for combining RNA molecules and propagating them outside of the plant lag far behind those available for DNA.

36/ Corn as High as a Geneticist's Eye. The Economist, Nov. 19, 1983. p. 105.

Direct methods of gene transfer in plants have also been proposed. Microinjection, similar to what is performed on animal cells, is one possibility. A single cell is held in place, usually by the slight suction of a very small pipette. An extremely fine needle is used to inject the foreign DNA directly into the cell's nucleus. Another example of a direct method of gene transfer is the free uptake of DNA by protoplasts, similar to methods used on bacteria. When a cell has been specially treated for cell wall removal, the cell membrane becomes "leaky" or permeable to DNA outside the cell. A certain small percentage of cells so treated will take up and incorporate the external foreign DNA into the cell's chromosomes.

II. APPLICATIONS

A. AGRICULTURAL PRODUCTS VIA TRADITIONAL AGRICULTURE

1. Disease and Pest Resistance

Pest and pathogen damage results in a significant loss in U.S. and world crop production. For example, although farmers apply expensive agricultural chemicals to prevent disease and insects in corn, approximately $4 billion of the world corn crop is lost each year. 37/ Plant pathologists and breeders are using the techniques mentioned in the previous chapter to develop new ways of combatting crop diseases and pests.

Plant cell and tissue culture is used in a number of important species to obtain pathogen free plants. Bacterial and fungal infection can be eliminated by surface sterilization of the plant tissue before placing it in culture. Plants derived from uncontaminated cultures will be free of these pathogens. To obtain virus free plants, the plant growing tip (or apical meristem), which is often not infected by virus, is used for tissue culture. However, since virus free plants are not immune to infection, exposure to viruses can lead to reinfection. New virus free plants would probably have to be purchased by the grower every few years to maintain maximum yields. Virus free plants have been used to increase the yield of strawberry plants.

37/ The New Plant Genetics. Report by L. William Teweles, and Company, Milwaukee, WI. Dec. 1983.

Scientists at the Agriculture and Forestry Experiment Station at West Virginia University are using cell culture and plant regeneration techniques in an attempt to save the American chestnut tree. At the present time, only about 50 mature trees are left in the United States, all of which are diseased. The chestnut blight is caused by a fungus which spreads via spores carried by rain, wind, birds and insects. The fungal growth encircles the tree, eventually preventing the flow of sap. Even though "new trees continue to sprout from the unaffected roots of the diseased chestnuts, they rarely reach seed-bearing age before they, in turn, are struck by the blight." 38/ The disease was first observed in New York in 1904. Before that time, "chestnut trees made up one-fourth of the eastern hardwood forests. Some trees were seven feet in diameter and reached heights of 100 feet and more." 39/ Cell culture and plant regeneration techniques will become useful when a blight resistant chestnut tree is found or developed. Such techniques will be used to rapidly propagate large numbers of resistant trees. "Seed-grown offspring produced by combining the genes of two parent trees, one resistant and one not, probably would not have the resistant trait." 40/ Attempts are being made to develop blight resistant trees by crossing the American chestnut with the naturally resistant Chinese and Japanese species.

38/ Chestnut and Redwood Trees Successfully Cloned. Bioscience, v. 32, Apr. 1982. p. 252.

39/ Ibid.

40/ Ibid.

Somaclonal variation is being used in a variety of species as a method of generating plants resistant to disease. Scientists at Kansas State University and Montana State University have found potato somaclonal variants that were less susceptible than the parent variety to damage by the fungus which causes early blight. Potato variants were also found that are resistant to late blight, "the disease which caused the Irish potato famine of the 1840s, and is still a major threat to commercial potato farming." 41/ In addition, protoplast fusion has been tried in efforts to transfer disease resistance genes from, for example, the tomato to the potato. 42/

Variability produced by cell culturing techniques can be used to screen for regenerated plants resistant to disease. Screening for resistance to eyespot disease in sugarcane was performed by treating plants regenerated from culture with the toxin produced by Helminthosporium sacchari, the causative agent of the disease. 43/ Fifteen to twenty percent of the regenerated plants were resistant to the disease. Other experiments of this nature in sugarcane developed plants resistant to Fiji disease and downy mildew; some plants also had increased sugar content. "Although no reports have appeared to date on the stability of these traits through sexual crosses, they have been maintained through several generations of vegetative propagation." 44/

Screening of this sort can also take place at the cultured cell level. In this case, plant cells are grown on a medium "supplemented with a lethal

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41/ Shepard, The Regeneration of Potato Plants from Leaf-cell Protoplasts, p. 154.

42/ Ibid., p. 166.

43/ Chaleff, R. S. Isolation of Agronomically Useful Mutants from Plant Cell Cultures. Science, Feb. 11, 1983. p. 678.

44/ Ibid.

concentration of the disease toxin. Consequently, this procedure is applicable only in cases in which a toxin produced by the microbial pathogen is primarily responsible for the disease symptoms." 45/ This approach was attempted with the wildfire disease of tobacco, caused by the bacteria Pseudomonas tabaci which produces a toxin thought to cause the disease. Regenerated plants resistant to the disease were produced, and the resistance trait was passed on to the progeny in typical Mendelian fashion. However, the attempt was only partially successful. Although the experiment yielded plants that were insensitive to the toxin, they were still susceptible to other deleterious effects of P. tabaci infection. 46/

Most plants have natural mechanisms for resisting pests and diseases. Adjacent leaves in the sugar maple, for example, "differ quantitatively in many chemical and physical traits important to insects trying to eat them. One group of leaves may be high in tannins and other metabolites that are toxic to insect herbivores while another section may have below-normal concentrations of nitrogen containing nutrients desired by the insects. The concentrations of these materials may change on a regular basis or in response to destruction by insects." 47/ By using such a strategy, the insect is forced to move frequently about the plant, thus exposing itself to predators.

Tomato and potato plants produce proteinase inhibitors in response to insect attack. The inhibitors, which interfere with the insects' digestion of plant, seem to be regulated by a "wound hormone" called proteinase inhibitor-inducing

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45/ Ibid.

46/ Ibid.

47/ Maugh, Thomas H. Exploring Plant Resistance to Insects. Science, May 14, 1982. p. 722.

factor. This hormone may also stimulate defenses throughout the rest of the plant. 48/

Many other chemicals are involved in the defense mechanisms of other plant species. Limonoids, found in citrus fruits, stunt the growth of several important agricultural pests. Chemicals which inhibit molting and chitin* synthesis have been found in the tropical medicinal shrub Plumbago capensis. 49/ Proposals for making use of such chemicals range from enhancing their production within plants by using genetic engineering, to manufacturing them via fermentation for external application like today's chemical pesticides. Schemes involving alteration of the plants natural defenses, however, must be approached with caution. "Plants generally produce defensive substances only in response to an attack because that production represents a drain on their energy reserves and their synthetic capacities. Increased resistance to insects and other pests can come only at the cost of a decreased yield or decreased production of biomass. If that decrease is more than the loss resulting from infestation, the process becomes self-defeating." 50/

Researchers are also trying to develop better methods of detecting plant disease. Scientists at the American Type Culture Collection in Rockville, Maryland, have developed monoclonal antibodies* to detect ten different plant

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48/ Ibid.

49/ Ibid.

50/ Ibid.

*Chitin forms the hard outer covering of insects.

*Monoclonal antibodies are defined as: "Homogeneous antibodies derived from a single clone of cells." (Commercial Biotechnology: An International Analysis. Office of Technology Assessment. Washington, D.C. U.S. Govt. Print. Off., Jan. 1984. p. 593.)

pathogenic viruses. 51/ Monoclonal antibodies are very specific, usually recognizing only one chemical structure, or what is called an antigenic site. However, some pathogens, such as viroids, are not suitable for monoclonal antibody detection since they do not have "antigenicity." Scientists at USDA have developed a method using recombinant DNA which can detect potatoes infected with the viroid causing potato spindle tuber disease. 52/ The disease is very difficult to eliminate from a field once it has become established. Methods such as this would allow the infected stock to be identified and discarded before planting would occur.

Other methods of agricultural pest control under investigation involve the manipulation of organisms, other than the crop plant, which can have adverse effects on pests. "Approximately 1500 naturally occuring microorganisms or microbial by-products have been identified as potentially useful insecticidal agents." 53/ Several microbial insecticides are already being marketed after having been approved by EPA. Because microbial insecticides are highly specific, they lack the problems associated with the extensive use of broad range chemical pesticides. However, their high cost of production and high specificity limits their general applicability. The Bacillus thuringiensis toxins are probably the most highly characterize microbial insecticidal agents. B. thuringiensis subspecies kurstaki produces a toxin which kills the leaf eating larval form

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51/ Gross, Michael. Tissue Culture, MCAs, DNA Probes for Plant Disease Diagnosis and Therapy. Bio/Technology, Dec. 1983. p. 859.

52/ Marx, Jean L. Agricultural Applications of Genetic Engineering. Science, June 18, 1982. p. 1306.

53/ Miller, L. K., A. J. Lingg, and L. A. Bulla. Bacterial Viral and Fungal Insecticides. Science, Feb. 11, 1983. p. 715.

of moths. The toxin made by B. thuringiensis subspecies israelensis kills mosquitoes and blackflies.

Viruses and fungi have also been commercially developed as pesticides. 54/ Other methods of biological pest control include the use of insects, such as wasps which parasitize the eggs of many harmful insect species, the release of selected frogs and birds which eat pests, and the use of pheromones and hormones to attract injurious insects into traps. 55/

Soils in many areas seem to have the ability to suppress the development of certain plant diseases. This property has been attributed to many factors: acidity, salinity, the proportion of clay or minerals in the soil, and biological components of the soil, such as the presence of certain types of root colonizing bacteria. 56/ The experimental application of such root colonizing bacteria, or rhizobacteria, to either seed or roots has resulted in significant increases in plant growth and yield. 57/ Rhizobacteria seem to exert their effects by either displacing the deleterious organisms on the plant root or by producing antibiotics that inhibit plant pathogens. Researchers are currently investigating the relationship between rhizobacteria and plants with the hope that alterations in such bacteria through genetic engineering could lead to substantial increases in crop yields.

54/ Ibid.

55/ Watanabe, Myrna. Biological Pest Control: New Chinese Export. Bio/Technology. May 1983. p. 246.

56/ Schroth, M. N., and J. G. Hancock. Disease-Suppressive Soil and Root Colonizing Bacteria. Science, June 25, 1982. p. 1376.

57/ Ibid.

2. Herbicide Resistance

Approximately 420 million pounds of herbicides are used each year by U.S. farmers to kill weeds that compete with crop plants. 58/ Since herbicides can also kill many crop plants, they may only be used on resistant crops. However, residues of herbicides such as atrazine, for example, often can remain in the soil. When an atrazine susceptible crop like soybeans is rotated into a field previously used for atrazine-resistant corn, the remaining residue of the herbicide can dramatically reduce the soybean yield. 59/ Often amounts of herbicides insufficient to achieve satisfactory weed control are placed on corn so that the following year the soybeans are not poisoned, thus reducing the potential yield of both crops.

Scientists at Michigan State University have located the gene for atrazine resistance to the chloroplast, the site of photosynthesis in the plant. Atrazine destroys susceptible plants by disrupting photosynthesis, the process by which plants convert sunlight to chemical energy. The gene for atrazine resistance has been isolated, characterized, and its expression has been achieved in bacteria. Inserting the gene into the chloroplasts of susceptible plants, however, may prove to be a difficult problem since the Ti plasmid inserts its genes only into the plant cell nucleus and not into chloroplasts. A solution to this problem could be protoplast fusion between an atrazine resistant plant (with its nucleus inactivated) and the desired crop plant. Researchers in "a half dozen laboratories around the world" are now trying to perform this experiment using an atrazine resistant weed, the blacknight shade, and the potato. 60/

58/ Genetic Engineering of Plants: Agricultural Research Opportunities and Policy Concerns. National Academy Press, Washington, D.C., 1984. p. 40.

59/ Ibid.

60/ Ibid.

Scientists at Calgene, Inc., a genetic engineering firm located in Davis, California, have isolated a gene which confers resistance to "Roundup," an herbicide developed by Monsanto. Calgene is hoping to develop "Roundup" resistant plants, specifically cotton, by 1989. "Cotton farmers, who spend about $150 million a year on cotton seed must use Roundup sparingly because it kills cotton plants as well as weeds." 61/

Many soil bacteria can enzymatically degrade herbicides. Provided these bacterial genes could be isolated and transferred into plants, many crops could potentially be made resistant to a variety of herbicides. Another approach would be growing cultured plant cells in the presence of herbicides; cells which survive and regenerate into adult plants would hopefully retain and express the herbicide resistance trait. However, Sheldon Krimsky, of Tufts University, has expressed what many feel to be justifiable objections to the development of herbicide resistant plants. "If we create food crops with herbicide resistance, are we not going to reinforce the use of herbicides? Are we not going to reinforce greater chemical use in food production at a time when people are increasingly questioning the agricultural use of chemicals?" 62/

3. Improvement Of The Protein Content In Seeds

The storage proteins of cereals (such as wheat, corn, oat, etc.) are deficient in amino acids essential to human and animal nutrition. Amino acids

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61/ Hughey, Ann. More Firms Pursue Genetic Engineering in Quest for Plants with Desirable Traits. Wall Street Journal, May 10, 1983. p. 60.

62/ Hilts, Philip J. The Test Tube Babies of Agriculture. Washington Post, Apr. 30, 1983. p. A8.

are the basic components of protein. Plants are able to synthesize all twenty amino acids required for protein production. Most animals, including humans, are unable to synthesize ten of the amino acids which must, therefore, be supplied in the diet. In order to provide a more balanced source of amino acids without the need for supplementation from outside sources, scientists are trying to improve the amino acid content of seed proteins.

The protein portion of a grain kernel is a collection of numerous related proteins all deficient in some of the essential amino acids and each encoded by a separate gene. In order to make a significant improvement in the overall quality of the stored protein, many, if not all, of the genes would have to be individually modified. However, within the kernel, all the storage proteins seem to be precisely folded together into the same type of three dimensional shape, what biochemists call protein secondary structure.* "Attempts to alter the genomic coding sequence of [these] proteins by genetic engineering must take into consideration the possible effect of amino acid changes on protein secondary structure, since the stability and accumulation of the [storage] proteins during embryogenesis may well be due to protein structural features." 63/

The introduction of "balanced amino acid" proteins from other plants such as legumes is another possible method of improving the protein content in grain seeds. However, the stability and structural problems mentioned in the previous paragraph would also have to be considered. An additional complication in this case, however, is the accurate timing and level of the new protein gene's expression. The new gene should only be active in the seed,

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63/ Barton and Brill, Prospects in Plant Genetic Engineering, p. 673.

*Secondary structure: a protein's primary structure refers to its sequence of amino acids; secondary structure refers to the type of configuration formed by the amino acid chain through intramolecular bonding.

and only during its development and maturation. Expression elsewhere in the plant would be an unnecessary waste of the plant's energy and resources, probably resulting in decreased seed yield. Scientists at the University of Wisconsin and Agrigenetics, a Colorado based seed company, have succeeded in transferring a bean storage protein gene, called phaseolin, to tobacco plants by using the Ti plasmid. 64/ The gene was expressed at low levels (less than one percent) in the stems and leaves of the regenerated tobacco plants. This is the first reported success in obtaining a foreign plant gene's expression in a whole plant.

4. Increase Yield

Micropropagation promises to greatly improve yield in oil palms. The oil palm is probably the first high value crop which has benefited from micropropagation. "In commercial terms, oil palms has been one of the world's outstanding successes of the past two decades. In Malaysia, the world's leading producer, oil palm is beginning to vie with rubber as a cash crop." 65/ Palm oil is used in soaps and increasingly as an oil in food products. It can only be propagated by seed or by micropropagation. When grown from seeds, oil palms show considerable genetic variation, and differ greatly in vigor, productiveness and resistance to disease. However, "these traits are often not expressed until the palms are ten years old, and the commercial life of a palm may be as

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64/ Murai, N. et al. Phaseolin Gene from Bean is Expressed After Tranfer to Sunflower via Tumor-Inducing Plasmid Vectors. Science, Nov. 4, 1983. p. 476.

65/ Tudge, The Future of Crops, p. 549.

long as 60-70 years. . . . Selecting parent palms and breeding new hybrids can take decades." 66/

In contrast, micropropagated lines of oil palm are genetically uniform. "Yield increases of 30 percent from first selections have been estimated. Better selections could conceivably double yield, while production costs are decreased through use of micropropagated palms that are easier to harvest and maintain. Oil type and quality can also be controlled to increase value and to extend the range of industrial application. The current seedling plantations of oil palm will almost certainly be progressively replaced by clonal micropropagated material over the next 20 years." 67/

Methods for the micropropagation of coconut palms are currently under development. The first clonal coconut plantlet was produced in March 1983 in a British laboratory after eight months of culture. 68/ Cloned coconut palms could increase the yield of coconuts by five fold over unselected stock. "In Kerala, India, an important producer of coconuts, the average yield is 35 nuts per palm per year. Yet single elite palms have been identified which produce yield of over 400 nuts per year. It will soon be possible to clone such superior palms." 69/ The economic impact of the development of asexual propagation methods for coconut could be greater than that for oil palm.

Yield, of course, can be theoretically increased by eliminating those external factors which stress the plant. Sources of stress are diseases, pests,

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66/ Branton, Richard, and Jennet Blake. A Lovely Clone of Coconuts. New Scientist, May 26, 1983. p. 554.

67/ Aynsley, Diotz, and Kidd, Micropropagation Enters a New Commercialization Phase, p. 167.

68/ Branton and Blake, A Lovely Clone of Coconuts, p. 556.

69/ Ibid., p. 557.

and competition with "weeds," as mentioned earlier in this chapter. Other sources of stress, which will be discussed in the following sections, are drought, salinity, temperature or lack of a substance required for plant growth, such as nitrogen. Another example is atmospheric CO , which the plant converts into carbohydrates through the processes of photosynthesis.

Intuitively, it seems that an increase in photosynthetic rate would correlate with an improved crop yield. However, such a connection is not easily demonstrated. "Although it has not been selected for directly by plant breeders in the past, one might have expected some indirect selection to have taken place, given the central role of the photosynthetic process. However, there is no evidence of this having occured." 70/ None of the modern high yielding cultivars of wheat, corn, sorghum, millet, sugarcane, cotton or cowpea have higher photosynthetic rates than either older cultivars or wild progenitors. "In fact, the highest rates for four of these crops are found among the wild relatives." 71/

Factors which seem to have increased yield in previous conventional breeding programs are the following: (1) reductions in stems, leaves, roots, and reserves; (2) changes in the crop's life cycle, allowing a longer duration of grain growth; (3) increased rates of storage, possibly influenced by improved vascular transport systems or increased enzyme capacity in synthesizing storage proteins; and (4) increased transfer of the plant's nutrient reserves into the harvested grain. 72/

70/ Evans, L. T. Raising the Yield Potential: By Selection or Design? In: Genetic Engineering of Plants: An Agricultural Perspective. Kosuge, Tsune, Carole P. Meredith and Alexander Hollaender, eds. New York, N.Y., Pleumn Press, 1983. p. 381.

71/ Ibid.

72/ Ibid., p. 378-381.

The yield potential for a crop is an extremely complex characteristic. "Crop yield is the ultimate outcome of the whole life cycle of the crop, and of the rates, durations and interlinkages of many processes at all stages of development, any one of which may be limiting to yield in a particular environment. No one process provides the key to greater yield potential, and the yield of different cultivars or hybrids at one site may be limited by quite different processes. . . . Thus, crop physiologists are not in a position to specify desirable single characters which are touchstones for greater yield potential and which have a simple genetic basis for the ready application of genetic engineering techniques." 73/

5. Nitrogen Fixation

Nitrogen is incorporated into a large variety of biological molecules and is a fundamental constitutent of proteins. Since it is essential for plant growth, the supply of nitrogen is a major determining factor in crop productivity. While nitrogen is abundantly present in the environment, it is usually found in a form which is not useful to plants. Industrial manufacture of the 16 million tons of nitrogen fertilizer used annually in the United States is an expensive process, requiring 608 billion cubic feet of natural gas. American farmers spend approximately $1 billion a year on nitrogen fertilizer for the corn crop alone. 74/

73/ Ibid., p. 385.

74/ Brill, Winston J. Agricultural Microbiology. Scientific American, Sept. 1981. p. 199.

Certain microorganisms are able to reduce atmospheric nitrogen (N_2) directly to ammonia, a usable form of nitrogen for plants, via a process called nitrogen fixation. Leguminous plants (such as soybean, alfalfa, peanut, bean, pea, and clover) do not require fertilizer since they obtain their nitrogen through a symbiotic association with the nitrogen-fixing bacteria Rhizobium. Through a complex infection process, these bacteria form specialized structures called nodules on the roots of legumes. Within the highly differentiated root nodules, the plant provides for the bacteria with carbohydrates and the bacteria supplies nitrogen for the plant.

The formation of nodules requires the presence of specific genetic information in both the bacterial and host plant cells. The nitrogen fixation (nif) genes of the bacteria Klebsiella pneumoniae have been studied extensively and this information is being used to understand the more complex and agronomically important symbiotic process of Rhizobium. K. pneumoniae has a contiguous cluster of seventeen genes organized into seven distinct units, each of which is separately regulated. Except for one protein, the function of the plant genes required for symbiosis have not yet been determined. This plant protein, called leghemoglobin, protects the bacterial enzyme nitrogenase from oxygen. The oxygen sensitive nitrogenase is the enzyme directly responsible for N_2 reduction to ammonia.

In areas where Rhizobium is indigenous to the soil, the farmer only needs to plant the legume. Where the bacteria is not present, the farmer inoculates the soil or coats the seed with Rhizobium and nitrogen fertilization is not necessary. Scientists are attempting to improve the efficiency of nitrogen fixation in legumes through genetic engineering.

Agrigenetics Corporation, of Boulder, Colorado, has licensed from Oregon State University a section of DNA that improves the efficiency of nitrogen

fixation in Rhizobium. 75/ 76/ The DNA contains the hydrogen uptake or hup genes which code for the enzyme hydrogenase. Bacteria produce hydrogen as a by-product of nitrogen fixation. About 30-40 percent of the energy used in nitrogen fixation is lost in the production of hydrogen. The enzyme hydrogenase allows the bacteria to recycle part of the hydrogen produced in the nitrogen fixation reaction thus reducing the loss energy. Only 25 percent of Rhizobium strains associated with soybeans have hup genes. The strains associated with alfalfa and clover do not produce hydrogenase at all. "Agrigenetic researchers note that soybeans may accumulate 15 percent more dry matter and 25 percent more nitrogen if they are inoculated with Rhizobium containing a hup system than can soybeans associated with the bacteria lacking the capacity for hydrogen uptake." 77/

Agrigenetics plans to transfer the hup genes to Rhizobium strains that are particularly active in fixing nitrogen, eventually marketing the bacteria as a soil inoculant. Such preliminary experiments have already been performed. However, "at present, a major obstacle prevents the engineering of competitive soil bacteria; laboratory modified soil organisms lose their ability to compete, and tend to disappear when reintroduced into the soil. Once a soil bacterium is isolated and modified in any of a variety of ways--even if it is grown under new conditions--and then returned to the soil, it is weakened or attenuated by comparison with other native strains." 78/ Agrigenetics is currently working on ways to eliminate this problem.

75/ Agrigenetics Get Rights To Genes That Improve Nitrogen Fixation. Genetic Technology News, Sept. 1983. p. 5.

76/ Netzer, William. Agrigenetics Targets Bacteria For Improvement Via HUP Genes. Genetic Engineering News, Nov./Dec. 1983. p. 39.

77/ Ibid.

78/ Ibid.

Other laboratories are focusing their efforts on extending nitrogen fixing abilities to plants other than legumes, such as the cereals. Winston Brill, of the University of Wisconsin and Cetus Madison Corporation is working with another nitrogen fixing bacteria, Azotobacter, in an attempt to supply corn with nitrogen. 79/ Azotobacter does not form natural symbiotic relationships, and ordinarily makes only enough nitrogen for its own growth. Brill is trying to modify both the bacteria and the corn plant in his experiments. He has isolated bacteria which excrete excess nitrogen, and is attempting to breed corn plants that are able to meet the energy needs of the bacteria. His laboratory has developed corn plants that obtained up to one percent of their total nitrogen from Azotobacter. 80/

Perhaps, a more ambitious goal is the introduction of nitrogen fixation genes directly into plant cell DNA. However, this approach has a number of obstacles: (1) the bacterial nif genes are regulated differently than the genes of higher organisms such as plants; (2) the nitrogenase enzyme has extremely high energy requirements which the plant may not be able to provide; (3) nitrogenase is inhibited by oxygen, which is present in relatively high levels in plant cells; and (4) nitrogen fixation requires more iron and molybdenum than are normally found in plant cells.

79/ Genetic Engineering of Plants: Agricultural Research Opportunities and Policy Concerns. p. 49.

80/ Ibid.

6. Resistance to Physical Stress Factors

Plants grown outdoors are often prevented from expressing their full genetic potential for yield because of stress factors present in their environment. One way to assess the effects of environmental stress is to compare average yields and record yields of the major crops. 81/ In 1975, the record yields of eight major crops were three to seven times higher than the average yields. "Corn, for example, yielded 4,600 kilograms per hectare on average but had a record yield of 19,300 kilograms per hectare." 82/

The difference between average and record yields is attributed to environmental stress factors. Disease, weed and insect losses are estimated to depress potential yield by 4.1, 2.6, and 2.6 percent respectively. 83/ Unfavorable physical environments, such as drought, temperature, etc., account for an estimated 69.1 percent of yield loss. 84/ The mechanisms by which plants cope with stress factors are just beginning to be understood. Scientists are attempting to use this information along with recombinant DNA techniques to improve the yield of crop plants. The appropriate genes will have to be identified and their function determined before plants with improved stress resistance can be genetically engineered.

The advances obtained in agriculture production have relied in part on the availability of water and the energy required for irrigation. "Water that is

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81/ Boyer, J. S. Plant Productivity and the Environment. Science, v. 218, Oct. 29, 1982. p. 443.

82/ Ibid.

83/ Ibid.

84/ Ibid.

usable for irrigation must increasingly be shared with municipal and industrial users, who offer larger economic returns (estimated at 50 to 1) than can be obtained from irrigation. . . . Considering competing needs. . . , it is likely that no more than 10 percent of U.S. agricultural land will be irrigated. Therefore, irrigation can only be a partial solution to the water resource problem." 85/

Irrigation also brings with it increased concentrations of salt in the soil. The amount of salt in heavily irrigated soils is becoming detrimental to many crops in certain areas of the country. According to the California Department of Conservation, more than 1.5 million acres of highly productive farmland (mostly in the San Joaquin and Imperial Valleys) suffer reduced yields from soil salinity, resulting in an estimated $30 million in annual crop losses. About 25,000 acres of prime farmland are being taken out of production every year because of this problem. Salty soil causes damage by drawing water out of living material, such as root cells, eventually killing the plant. The effect is essentially the same as what happens to the plant during a drought.

Various organic compounds have been known to accumulate in plants following periods of drought. "Seeds, which represent a severely dehydrated state, have been found to be a rich source of these compounds." 86/ In 1982, scientists at the University of California, Davis, reported the isolation of a gene (osm) for the production of one such compound which helps protect the

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85/ Ibid., p. 444.

86/ Strom, A. R., D. LeRudulier, M. W. Jakowec, R. C. Bunnell, and R. C. Valentine. Osmoregulatory (Osm) Genes and Osmoprotective Compounds. In: Genetic Engineering of Plants: An Agricultural Perspective. Kosuge, T., C. P. Meredith, and A. Hollaender, eds. Plenum Press, New York, N.Y., 1983. p. 41.

bacterium Salmonella from a lack of water. 87/ The gene was subsequently transferred to Klebsiella, a nitrogen fixing bacterium normally very sensitive to the lack of water. The gene rendered Klebsiella much less sensitive to high salt concentrations. The researchers at Davis are planning to transfer the osm gene to Rhizobium japonicum, the symbiotic nitrogen-fixing bacterium associated with soybeans, which is also very sensitive to drought conditions. Eventually, they hope to apply this technology in higher plants including the major crops.

Protoplast fusion is being used to develop salt tolerant varieties of rice. Wild varieties of rice, which do not have the high yields of cultivated varieties, are able to grow under saline conditions. "For many people of Asia rice is a subsistence crop: what counts is not so much spectacular yield or cosmetic qualities to impress buyers but the ability to withstand local conditions, and salinity is one condition found all too frequently." 88/ A sexual cross between the two rice varieties is not possible because they are not capable of interbreeding. Fusion of rice protoplasts have produced laboratory hybrids, but regeneration of a whole hybrid rice plant has not yet been achieved. 89/

Conventional breeding techniques are also being used to develop salt-tolerant crops. Salt-tolerant species of tomato collected from the Galapagos Islands were cross bred with a commercial variety by another group of scientists at the University of California, Davis. Eventually, after "several successive backcrosses, (to incorporate desirable characteristics such as size and color)"

87/ LeRudulier, D., and R. C. Valentine. Genetic Engineering in Agriculture: Osmoregulation. Trends in Biological Sciences, Dec. 1982. p. 431.

88/ Tudge, The Future of Crops. p. 551.

89/ Ibid.

the cross-bred tomatoes were grown with irrigation water having 70 percent of the salinity of seawater. 90/ While the commerical variety did not even survive, the cross-bred variety flowered and produced "fruit about the size of large cherry tomatoes, with flavor components much more intense than those of the commercial parent." 91/

Frost damage to crops caused by temperatures at or below 32 F. cost U.S. agriculture from $2 billion to $4 billion each year. 92/ Scientists at the University of California, Berkeley and Advanced Genetic Sciences, Greenwich, Connecticut, are currently working on a method of preventing frost damage in crop plants. They have used genetic engineering techniques to alter the bacterium Pseudomonas syringae, which is thought to be involved in frost formation plants. Molecules on the bacteria's surface react with water at low temperatures to form ice crystals. Pseudomonas syringae normally grows on plant surfaces. Without the bacteria, some plants "resist frost formation and survive at temperatures as low as 23 F. . . . Farmers often try to kill Pseudonomas with chemical sprays. But, sprays are not especially effective . . . [and] must be applied as often as every three days." 93/

The genetically engineered Pseudomonas is missing one gene, which causes changes in the surface characteristics of the bacteria, preventing the organization of water molecules into ice. The scientists are ready to test the

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90/ Epstein, E., J. D. Norlyn, D. W. Rush, R. W. Kingsbury, D. B. Kelley, G. A. Cunningham, and A. F. Wrona. Saline Culture of Crops: A Genetic Approach. Science, Oct. 24, 1980. p. 402

91/ Ibid.

92/ Schneider, Keith. First-Ever Release of a Lab-Created Life Form. California Journal, v. 15, Feb. 1984. p. 52.

93/ Ibid., p. 51.

altered bacterium outdoors on a test plot of potato plants, and on June 1, 1983, the National Institutes of Health (NIH) authorized the experiment to take place. However, a coalition of public interest groups, including the Foundation on Economic Trends. Environmental Action, Inc., and the Environmental Task Force, have delayed the experiment by suing NIH and the Recombinant DNA Advisory Committee (RAC)*, charging that their procedures for genetic engineering oversight are inadequate. The suit alleges that NIH has violated several Federal laws in addition to failing to abide the National Environmental Policy Act by neglecting to file an environmental impact statement. The coalition's suit also asserts that NIH and RAC "lack the necessary scientific expertise to evaluate the risk of such experiments." The experiment has been rescheduled for May 1984, following the Federal court's decision which is due in the spring of 1984.

7. Other Characteristics

Genetic engineering and tissue culture could also be used to improve other characteristics of agriculture products such as color, texture and flavor. For example, several companies are trying to develop improved tomatoes by using recombinant DNA techniques. ARCO and the H. J. Heinz Company are attempting to "double tomato solids as well as to make the tomato more disease-resistant and better adapted to field conditions." 94/ Campbell Soup Company and DNA

94/ New Plant Genetics to Change Grocery Store in Next Century. Genetic Engineering Letter, v. 3, Dec. 10, 1983. p. 1.

*RAC advises NIH on the safety of recombinant DNA research but does not have any regulatory authority.

Plant Technology, a genetic engineering firm based in New Jersey, are working on improving the tomato as well.

Approximately 70 percent of the tomatoes grown in the United States are processing tomatoes, used in ketchup, soup, sauces, pastes, and other canned tomato products. Consumption of tomatoes has increased by 15 percent in the last 10 years, mainly due to the increased popularity of convenience foods. Processors are primarily interested in the fleshy or bulk parts, which is only 5 percent of the tomato, the remaining 95 percent being water. Increasing tomato solids "from 5 to 6 percent would add almost $80 million to the annual value of processing tomatoes in the United States alone." 95/ Other characteristics targeted for improvement "include high acid content (to add flavor to processed tomatoes), a square shape (for more economical shipping) and a uniform candy apple red color." 96/

B. AGRICULTURAL PRODUCTS VIA PLANT TISSUE CULTURE

Like bacterial and mammalian cells, plant cells can also be cultured to produce marketable products. Many academic and industrial laboratories are working to develop plant tissue culture as a viable alternative to traditional methods of obtaining plant products. Almost one quarter of all prescription drugs in the United States are derived from plants. For centuries plants have been a major resource of chemicals and are now used to produce gums, pesticides, perfumes, flavor additives as well as pharmaceuticals. These valuable chemicals are termed secondary metabolites because, unlike fats, proteins and carbohydrates

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95/ Ibid.

96/ Ibid.

(which sustain the plant from day to day), they have evolved specifically to affect other living things. Perfumes and coloring agents attract pollinators, drugs, pesticides, and some of the flavorings probably function to ward off predators.

Plant tissue culture has several advantages as an alternative route of natural product synthesis. These are:

(1) independence from various environmental factors, including climate, pests, geographical and seasonal constraints;

(2) defined production systems, with production as, and when required and at the amount required, hence giving a close control over market supply;

(3) more consistent product quality and yield;

(4) reduction in land use for 'cash crops;' and 97/

(5) freedom from "politically unstable third world countries where the government may decide to cut exports, raise prices arbitrarily, or grow something else next year." 98/

Chemical synthesis of secondary metabolites is another alternative, frequently less expensive than plant tissue culture if the product has a simple, well defined chemical structure. However, the synthetically derived products may not be judged to be of the same quality as the natural product. This is especially the case where quality is assessed by esthetic rather than functional values, such as in the flavor and fragrance industry. Often these products are composed of complicated mixtures of many different chemicals.

97/ Fowler, M. W. Commercial Applications and Economic Aspects of Mass Plant Cell Culture. In: Plant Biotechnology. S. H. Mantel and H. Smith, eds. Cambridge University Press, Cambridge, Gt. Br., 1983. p. 4.

98/ Curtin, Mary Ellen. Harvesting Profitable Products from Plant Tissue Culture. Bio/Technology, Oct. 1983. p. 650.

There are several technical and economic problems with using plant tissue culture in lieu of traditional plant extraction methods. The product generally must be of high value, at least $1,000 per kilogram, in order to justify the increased costs of development and production. Plant cell cultures are delicate and culturing methods are laboriously determined through trial and error.

Often, plant cells in culture do not produce secondary metabolites at all, presumably because some degree of differentiation is required. In culture, the cells have a tendency to aggregate or clump at the high densities necessary for adequate production. Aggregation tends to cause problems when the process is "scaled-up" into a larger reaction vessel since it disturbs the dynamics of fluid circulation. "Cells on the outside of a clump and those on the inside do not differentiate in the same way, so the variety and relative abundance of cell types in the reactor may vary unpredictably from one experiment to the next." 99/

Cell immobilization frequently solves many of the aggregation problems associated with high cell density. The cells are entrapped in a bead or on a membrane and bathed in the culture media which is continuously drained off as the product accumulates. However, many plant cells do not excrete products but collect them internally in organelles or vacuoles. Certain chemicals such as DMSO or changes in pH can be used to make the cells permeable in order to release the secondary metabolite.

The first commercial product manufactured by plant cell culture was announced in July 1982 by Mitsui Petrochemicals Industries of Tokyo, Japan.

99/ Ibid., p. 634.

The product is shikonin, a very expensive red dye, which is also used in Japanese traditional medicine as an anti-inflammatory agent. Shikonin was originally extracted from the root of the Lithospermum erythrorhizon, or shikon plant. "Problems with conventional methods of shikonin production make the substance attractive as a candidate for plant tissue culture. Lithospermum plants must grow for 5-7 years before the shikonin concentration in their roots reaches 1-2 percent." 100/ Commercial quantities of the plant could not be grown in Japan, so the entire supply had to be imported from China and Korea. Since pure shikonin costs $4,500 per kilogram, it was worth while for Mitsui to consider plant tissue culture production methods.

Other potential plant tissue culture products are the alkaloids vinblastine and vincristine produced by the periwinkle plant, Catharanthus roseus. Both have been approved by the FDA for cancer treatment. Another is the steriod digoxin produced by foxglove, or Digitalis, which is used as a heart stimulant. Additional possibilities include the analgesic codeine, the antimalarial drug and flavoring agent quinine, the insecticide pyrethrin, and jasmine, a perfume ingredient.

100/ Ibid., p. 651.

CONCLUSIONS

Two factors are necessary to advance the present level of research discussed in this report and expand its potential applications: (1) an adequate source of research and training grant funding; and (2) an adequate source of plant genetic resources.

The U.S. Department of Agriculture (USDA) has been a long time supporter of research on traditional plant breeding. However, the advances in genetics described in this paper were primarily supported by grants over the past ten years from the National Institutes of Health (NIH) and the National Science Foundation (NSF). In 1978, the USDA established a competitive research grants program in order to attract scientists outside the USDA and the Agricultural Research Service into conducting research in these new areas which are of great importance to agriculture. In FY83, the program received $16 million and in FY84 $17 million for research mainly on plant science. The Administration asked for a major new initiative in FY85 of $28.5 million for new grants to promote agriculture related research in genetic engineering and biotechnology. The $28.5 million would be in addition to a new $4.5 million program for animal science and last years's $17 million for plant science, bringing the program's total to $50 million in FY85 if Congress agrees to the Administration's budget. As may be noted, these amounts are relatively small in comparison with other areas of research in the biomedical sciences.

Few graduate and post graduate students choose the plant sciences for their research projects because the lack of training grants and fellowships influences

them to study other areas of biology. In 1983, the NSF began a small five year experimental training program in the plant sciences which was intended to fund 20 new two-year post-doctoral fellowships in each of the next five fiscal years. In the first year of funding, the programs received applications from 196 well qualified individuals for the first 20 fellowships. NSF managed to find additional money and consequently funded 24 grants in FY83.

The USDA will initiate a new program in FY84 which will fund, on a competitive basis, graduate fellowships in four targeted areas of "national need" in agriculture research. One of the four areas is biotechnology, which will receive the majority (35%) of the $5 million made available for the first year of the program. The fellowships are intended to provide funds for approximately 300 outstanding new graduate students at universities with distinguished graduate teaching and research programs in agriculture. Provided that the program is cleared by the Office of Management and Budget, USDA expects to begin implementation of the program before the end of FY84.

Within the next decade, without concerted conservation efforts, many important plant varieties may become extinct. As modern high yielding varieties are developed and marketed, farmers usually stop growing the older low yielding local varieties which consequently die out. This is especially critical in the underdeveloped or third world countries, where most of the major crops originally evolved and where most of the remaining "wild" varieties are still to be found. The genetic basis for the popular improved varieties, however, is the now rapidly disappearing wild stock. Plant breeders have traditionally used wild varieties as a source of, for example, genes that provide resistance to plant disease.

New genes are constantly required by plant breeders, as is shown by the following partial list:

(1) Chinese cherry genes were used to save British orchards several years ago;

(2) Honduran maize saved the American corn crop, which was nearly wiped out in 1970 by its uniform susceptibility to leaf blight;

(3) the tomato industry could not survive without continual cross breeding with Latin American and Filipino strains;

(4) a variety of wild melon from India provided resistance to powdery mildew in a majority of California cantaloupes;

(5) a seemingly useless strain of wheat from Turkey conferred disease resistance to many commercial varieties, with an estimated annual benefit of $50 million;

(6) a Peruvian species transferred ripe rot resistance to American pepper plants;

(7) an Ethopian plant provided yellow dwarf resistance to barley which in turn was used to pass resistance on to experimental wheat strains; and

(8) a Middle Eastern relative of safflower conferred its rust resistance on to a Californian variety. 101/

Efforts are being made in many countries to provide "gene banks" in order to preserve the natural genetic diversity of important crop plants. Most plant seeds can be stored at 20 C for several decades. Some crops are more delicate and must be frequently "grown out" to maintain their viability. However, the reliability of banks has been questioned by individuals who are concerned about the possibility of electrical power loss, fire, floods, disease, or even lack of funds to maintain the banks. Other objections to gene banks include the fact that removing seeds from the environment prevents "the selective pressures

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101/ MacKenzie, Debora. Seeds of Conflict Over Food Genes. New Scientist, Dec. 22/29, 1983. p. 870.

of naturally mutating plant diseases and pests, genetic drift and [results in] the loss of invaluable information on the habitats and cultural practices associated with particular cultivars." 102/

There is also controversy over the location and consequently the control of gene banks. Third World countries complain "about the increasingly tight grip of Western seed companies on the genetic resources of the world's food crops." 103/ "Wheat genes originally taken from Libya and Afghanistan have been withheld from these countries where they no longer exist." 104/ The "gene hoarding" issue could potentially escalate into a global problem rather than only a developed-underdeveloped altercation. "By 1985, the less-developed countries are expected to overtake the industrial nations in the number of gene banks with long-term storage capacity." 105/ Ethopia apparently has taken a strong stand on genetic resources and no longer allows plants or seeds to leave its territory. This action "could affect coffee growers throughout the tropics--notably the Brazilians, who need coffee strains from the Ethiopian highlands to improve their crops. Ironically, Brazil is another genetically rich country considering a tough stance on the use of its resources." 106/

102/ Dahlberg, Kenneth A. Crop Germplasm Conservation. (letter) Science, v. 221, July 29, 1983. p. 414.

103/ MacKenzie, Seeds of Conflict Over Food Genes, p. 870.

104/ MacKenzie, Debora. U.N. Takes Control of World's Food Genes. New Scientist, Nov. 24, 1983. p. 558.

105/ Plucknett, D. L., N. J. H. Smith, J. T. Williams, and N. Murthi Anishetly. Crop Germplasm Conservation and Developing Countries. Science, v. 220, Apr. 8, 1983. p. 16.

106/ Timberlake, L., J. Tinker, B. Cheney, and J. McCormick. Saving the World's Genetic Bank. World Press Review, Dec. 1982. p. 35.

The issue becomes even more complex when one considers the fact that the areas where commercial crops are grown on a large scale for harvest and export are often located at a considerable distance from the regions that contain the wild species. "Bananas, wild in South Asia, are cultivated in the Caribbean and Central America; rubber, wild in South America, is cultivated in Southeast Asia; cocoa, wild in South America, is cultivated in West Africa. In each case, the country that must pay for conserving the wild genetic resource is not one of the countries that will benefit from improved crops." 107/

Genetic engineering and plant tissue culture techniques hold considerable promise for the future as powerful supplementary tools to traditional plant breeding. However, the anticipated advances and improvements in agriculture suggest that the two issues of funding and availability of genetic resources be considered.

107/ Ibid.

APPENDIX

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ANIMAL SCIENCES: ADVANCES IN REPRODUCTIVE AND HEALTH TECHNOLOGIES

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July 28, 1983

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OVERVIEW

Man has evolved in an environment containing other animal species which he depends on for food, clothing, recreation, and companionship. Animal products for human consumption provide over 98 percent of the vitamin B_{12}, in the average human diet, as well as 69 percent of the protein, 80 percent of the calcium, 67 percent of the phosphorus and vitamin B_6, over 50 percent of the vitamin B_2 and a substantial proportion of most other nutrients. 1/ The survival of human societies without their domestic animals would certainly be difficult, if not impossible, especially in nonaffluent cultures. 2/

As specified by the subcommittee, this paper is limited to recent advances in animal science and concentrates on two major areas: reproduction and health. The section on reproductive technologies begins with a discussion of artificial insemination, which has been used as an animal breeding tool in the United States since 1938. The impact of artificial insemination upon livestock productivity, particularly in the dairy industry, may serve as a model for the possible impact of the remaining techniques discussed in the section, which are not as highly developed. These techniques include embryo transfer, sexing of sperm and embryos, *in vitro* fertilization, twinning, cloning, nuclear transplantation and gene transfer.

1/ Pond, Wilson G., et al. Animal Agriculture: Research to Meet Human Needs in the 21st Century. Boulder, Colorado, Westview Press, 1980. p. 36.

2/ Seidel, George E., Jr., and Benjamin G. Brackett. Perspectives on Animal Breeding. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 4.

The second section discusses recent advances in biotechnology that hopefully will improve animal health. Diseases affecting livestock can cause major economic losses around the world and reduce food production at a time when both population and the demand for meat are growing. African cattle farmers, for example, lose $5 billion a year because large areas of land are infected with trypanosomiasis, or sleeping sickness, and cannot be used for cattle grazing.

Advances in biotechnology offer the promise of solving some of the current problems in animal health care. These advances, including the production of recombinant DNA and synthetic peptide vaccines to prevent disease, the development of monoclonal antibodies to diagnose infection and offer passive immunity, the creation of improved feed crops and additives to improve animal nutrition, and the large-scale manufacture of growth hormones to speed animal maturation, should help the farmer produce more food for more people.

I. REPRODUCTIVE TECHNOLOGIES

A. ARTIFICIAL INSEMINATION

In 1938, the technique of artificial insemination (AI) was introduced to the United States from Europe where it had been used primarily to control venereal disease in livestock. AI use in the United States, however, has mainly focused on the genetic improvement of domestic animals. For example, the U.S. dairy herd now numbers less than half what it was in 1950, yet it produces more milk. AI techniques are more highly developed for use in cattle than in other livestock species, and for a variety of reasons AI is much more frequently used in dairy cows than in beef cattle. Of the 11 million commercial dairy cows in the United States today, about 60 percent have been bred by AI. In contrast, of the 80 million beef cattle in the United States, only 5 percent have been bred using AI. 3/ This section will focus on the use of artificial insemination techniques in cattle and will also briefly describe its use in other livestock species.

Use in Cattle Production

There are many advantages to using AI in livestock reproduction, the greatest being the genetic improvement possible through careful sire selection. A bull in natural service may breed 25 cows per year over a lifetime of approximately 8 years, producing possibly 200 offspring. When AI is used, a bull,

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3/ Caras, Roger. Breeding A Revolution. Geo, v. 2, Aug. 1980. p. 85.

whose sperm is collected three times weekly over an 8-year period with each collection extended to breed approximately 200 cows, could ultimately produce 249,600 offspring. This conservative estimate is over 1,000 times what is expected from natural service. AI also allows for the rapid identification of high-quality sires. This may be accomplished in a single generation where it would usually take a lifetime to accumulate the same information using natural service. AI allows for a more complete and accurate record of livestock crosses and the resultant progeny's genetic worth. Such records are necessary for good reproductive management of a livestock herd and, when reported to central stations for processing, they provide valuable information for fertility and genetic research. AI can be used to overcome problems of breeding incompatibility that are due to, for example, injury, physical size, age, temperment or other factors. It also eliminates the need for the presence of dangerous bulls on dairy farms; dairy bulls are usually more excitable than beef bulls and must be handled with great caution. The use of AI has resulted in a significant reduction in the frequency of recessive lethal genes. Its use has also been instrumental in the control of certain diseases, especially venereal diseases. And AI is economical to use. The cost of using an outstanding sire through AI is less than through natural service and the value of the progeny produced exceeds the costs of semen and service. Finally, the success of AI as a breeding technique has provided the basis for the development of many other potentially useful techniques, such as in vitro fertilization and embryo transfer, which will be discussed in the following sections.

Bull semen is frequently collected using an artificial vagina, or AV. The AV is a stiff rubber hose which has been fitted with a soft rubber liner. Between the hose and the liner is a space containing warm water. One end of the AV is open and well greased, and at the other end is a flexible rubber

funnel which is attached to a collecting test tube. The bull is allowed to mount another animal, usually a castrated male, and a technician using the AV can collect the semen in less than 5 seconds. Alternatively, an electro-ejaculator may be used, eliminating the need for a mount-animal and enabling semen collection from injured and impotent animals and those unaccustomed to handling. Bull semen is collected two or three times a week, usually two ejaculations 10 or 15 minutes apart, yielding 10 to 30 milliliters of semen. The sample is examined under a microscope for sperm motility and with a spectrophotometer for sperm concentration.

A number of technical advances have occurred over the last 40 years in the collection, processing and storage of semen. Through the study of sexual behavior, methods of sexual preparation have been developed, along with an optimal schedule of semen collection. These efforts have resulted in an increase in the number of sperm cells harvested per sire per week from about 8-10 billion to about 30-40 billion. 4/ Methods of extending (or diluting) semen and preserving sperm also have been developed which permit more inseminations per ejaculation.

Following the sample's evaluation for sperm motility and density, it is diluted and cooled to 41 degrees F. After 3 to 4 hours, glycerin is added as an antifreeze to prevent the formation of harmful ice crystals which would rupture the sperm when the sample is later frozen. Next the sample is divided into .5 to 1 milliliter quantities and packaged in small plastic straws or glass ampules. Each straw or ampule contains from 10-15 million sperm, enough for one insemination. The straws or ampules are then frozen at -385 degrees F in liquid nitrogen. The freezing process kills up to one-half of the sperm cells

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4/ Foote, Robert H. The Artificial Insemination Industry. In: Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 19.

and tends to lower the fertility of the surviving cells slightly. However, the decrease in fertility is considered to be offset by the increased convenience of frozen semen, which may be stored indefinitely.

Before a cow is inseminated, it is necessary to determine if she is in estrus. Estrus is defined as the time during which the female will accept the services of the male and is capable of conceiving. It is cyclical in nature, the length and period varying among species. The cow is considered to be a polyestrous animal because she cycles continuously. (Estrus detection will be discussed in greater detail in the following section on embryo transfer.)

The most common technique for artificially inseminating a cow is the rectocervical method. The semen, which had previously been frozen in a straw or ampule, is thawed and inserted into the inseminator. A technician inserts one arm into the rectum of the cow and directs the inseminating tube through the vagina and cervix for deposition of the semen in the anterior cervix. Provided the cow was in estrus and the insemination was performed properly, a calf should be expected in approximately 283 days.

Dairy Cattle

The use of AI in the U.S. dairy industry is largely responsible for the increased milk production per cow mentioned at the beginning of this section. Between 1945 and 1975 the average amount of milk produced per dairy cow per year increased from approximately 2,000 kilograms (kg) to 4,700 kg. 5/ In 1945, 26.6 million dairy cows produced 54 billion kg of milk; in 1975,

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5/ Pond, Wilson G., et al. Animal Agriculture: Research To Meet Human Needs in the 21st Century. Boulder, Colorado, Westview Press, 1980. p. 117.

11.6 million cows produced 55 billion kg of milk. 6/ The feed that would have been required for maintenance of the 15 million cows which was not needed in 1975 is essentially feed saved and has been estimated at more than 1,000,000,000 bushels of corn annually. 7/ The increased milk production per cow has also resulted in decreased labor costs and facilities requirements.

The genetic improvement due to AI use in dairy cows is permanent and has been estimated to account for approximately 30 percent of the total increase in milk production. 8/ The balance is due to environmental factors such as feeding, housing and management. The importance of genetic superiority in dairy cows can be appreciated when one considers the fact that 400 pounds of blood must pump through a cow's udder for every pound of milk she produces. 9/ Thus, the construction of the udder, which is genetically determined, is of great importance. Superior dairy sires are usually identified by the milk production of their sister siblings and, of course by the milk production of their female progeny. Superior dairy cows average over 10,000 kg of milk per year with the record production of a single cow being over 25,000 kg of milk in one year. 10/

The National Cooperative Dairy Herd Improvement Program (NCDHIP) is a nationwide program for collecting, analyzing and disseminating information on dairy cattle performance. On the local level, the Dairy Herd Improvement

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6/ Ibid.

7/ Foote, Artificial Insemination Industry, p. 29.

8/ Ibid., p. 22.

9/ Caras, Breeding A Revolution, p. 88.

10/ Warwick, Everett James, and James Edward Legates. Breeding and Improvement in Farm Animals. McGraw Hill, 1979. p. 348.

Association (DHIA) collects performance data on each animal's milk production from the individual dairies. This information is sent to the Animal Improvement Programs Laboratory (AIPL) at USDA in Beltsville, Maryland which analyzes and incorporates it into the "USDA-DHIA Sire Summary List," published biannually for public use. The list is used by dairy breeders in selecting superior sires. NCDHIP is a cooperative program, financed largely by the dairy industry. The data provided by the program is considered to be impartial, since it is collected by disinterested DHIA officials and processed under high security conditions. AIPL's sire summaries are respected internationally because of the absence of commercial influence on their compilation and analysis of milk production data.

Not every State or region participates in the NCDHIP. In areas where dairying is scarce or only a part-time operation, a strong local DHIA does not exist. However, dairy cows enrolled in the the NCDHIP consistently outproduce the nonenrolled cows. In the May 1, 1977 to April 30, 1978 testing year, the NCDHIP animals produced 5,000 pounds more milk per cow, representing 52 percent more milk per lactation. 11/ The genetic gain that is due to the use of AI is predicted to continue increasing. There is no evidence at the present time that genetic variability is decreasing or that a plateau in milk production improvement has been reached.

The use of AI has been greater in dairy cattle than in other species because of their high value, regular contact with dairymen, the use of confined housing and the good results obtained with the use of frozen semen. Furthermore, milk yield and fat content are obvious traits for selection in dairy cattle. In other livestock species, the selection traits are not as obvious.

11/ U.S. Congress. Office of Technology Assessment. Impacts of Applied Genetics. Washington, U.S. Govt. Print. Off., 1981. p. 181.

Beef Cattle

Although the beef cattle industry is much larger than the dairy industry in terms of numbers of animals and their dollar value, less than 5 percent of its animals have been bred using AI. 12/ Natural stud service is usually more economical. Estrus detection in large numbers of cows scattered widely on the range is a significant barrier to AI application in beef cattle. Confined housing and the use of effective methods in estrus regulation would eliminate this problem.

Sheep Industry

Test programs using AI in sheep have not been particularly successful. The problems of handling animals on the range and difficulties in estrus detection remain as major barriers to AI application in the sheep industry. The use of AI is also limited by the poor fertility of frozen ram semen and relatively high labor costs per inseminated animal. The low cost of performance-tested rams available for natural service remains preferable to AI.

Dairy Goat Industry

The dairy goat industry has recently become interested in AI and has formed the "Coordinating Sub-Group for Dairy Goats" of the NCDHIP. Although dairy goats are housed in confined areas and AI is technically feasible, the insemination procedure is more difficult and the value per animal, labor costs, and estrus detection problems limit the potential application of AI.

12/ Foote, The Artificial Insemination Industry, p. 18.

Pork Industry

The increased use of confined housing in swine production has made AI more attractive to the pork industry. Although estrus detection is still a problem with gilts (young female swine), estrus in sows may be synchronized by the simultaneous weaning of litters. The fertility of frozen boar semen has been low, and when the female does conceive, the litter size tends to be smaller. Fresh semen generally gives better results when AI is used in swine. However, tested boars are available for natural service at relatively low cost. The concentration of sperm in boar ejaculate is very low and requires additional processing before freezing. The processing of boar semen still needs further research before a feasible AI method will become available for widespread commercial use in swine.

Horse Breeding

Horses are relatively easy to breed with AI because of size and anatomical structures. However, certain horse breed organizations are opposed to the use of AI and do not allow the registration of animals which have been bred using AI. As a result, commercial attempts to offer AI services to horse breeders have not been successful. Conception rates with frozen stallion semen are low; therefore, when artificial insemination is used on mares, fresh semen is usually employed. The period of estrus in the mare is considerably longer than in other species, lasting usually 6 to 8 days. (Estrus duration in the cow, ewe and sow is 16 hours, 30 hours and 44 hours, respectively.) Detection of ovulation in such a long estrous period is difficult and often necessitates multiple inseminations. AI can be used along with natural service for multiple inseminations in order to conserve the stallion. A stallion in high demand for natural service might otherwise exhaust his semen supply and conception rates would consequently decrease.

Poultry Industry

The entire turkey industry in the United States depends on AI since the selection of large amounts of white meat has produced birds which have difficulties in mating. Fresh semen is generally used in the poultry industry and can be rapidly collected. Frozen poultry semen is not as fertile and usually is not used. Large numbers of hens can be quickly inseminated using AI. Dilution methods for turkey semen have been developed, increasing the number of hens that may be mated to one male. Currently about 50 percent of hens are inseminated with diluted turkey semen. 13/

Economic Impact on Cattle Industry

The use of AI and careful sire selection has made a definite economic impact upon the dairy industry. It has been estimated that for each $1.00 invested in research, $50.00 has been returned to the economy. 14/ Approximately 7 million dairy cows and heifers are inseminated annually. Dr. Robert H. Foote, Cornell University, estimates that in 1977 the genetic improvement of dairy cows had resulted in an increase of 1,000 pounds of milk per cow. 15/ Assuming that the price of milk is $.10 per pound, Foote makes the following calculation: 7×10^6 cows x 1000 lbs milk/cow x $.10/lbs milk = $700,000,000. In other words, the increased genetic superiority of dairy cows was worth about $700,000,000 in milk for the dairy industry in 1977.

13/ U.S. Congress, Office of Technology Assessment, Impacts of Applied Genetics, p. 190.

14/ Foote, The Artificial Insemination Industry, p. 22.

15/ Ibid.

The cost of bull semen varies from a minimum of $3.00 per insemination to a few hundred dollars per insemination, the average being about $8.00. The cost of providing professional insemination service is about $5.00 per cow. 16/ Costs are approximately the same in the beef cattle industry. 17/

West Virginia University's Allegheny Highlands Project (AHP) has emphasized the selection and use of superior weight-gaining bulls as herd sires (using natural mating or AI) in studies on improving beef cattle production. AHP also included improved nutrition, health and overall management in their efforts on increasing the calf weaning weight (a standardized measurement at 205 days of age, adjusted for the sex of the calf and the age of the calf's mother). Increases in calf weights at weaning have averaged 10 pounds per year of participation in AHP. 18/ It has been estimated that if the increase in calf weight achieved by AHP participants were extended to all the calves in the State, $3,600,000 per year would be added to the income of West Virginia's beef producers when calf prices average $50.00 per 100 pounds (hundredweight). If AI were used, this $3,600,000 increased income could occur within 40 to 48 months. 19/

B. EMBRYO TRANSFER INDUSTRY

Embryo transfer consists of removing an embryo in an early developmental stage from its genetic mother's reproductive tract and transferring it to

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16/ Ibid., p. 31.

17/ Ibid., p. 35.

18/ Inskeep, E. Keith, and John B. Peters. Economic Benefits of Reproductive Management. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, Inc., 1981. p. 246.

19/ Ibid.

another female's reproductive tract for development and subsequent birth. 20/ In 1890, the first recorded embryo transfer was performed by Walter Heape, an English biologist, who succeeded in producing purebred Angoran rabbit young in a Belgian hare female. Few similar experiments were published until 50 years later, when embryo transfers in sheep were performed and reported. As one source notes, "A great amount of interest was generated in the late 1940s when livestock researchers felt . . . [embryo transfer] could be the female sequel of artificial insemination. Much--in fact too much--publicity was given to a program that did not materialize." 21/

Embryo transfer continued to be a laboratory tool used primarily to study the process of reproduction until the early 1970s, when European breeds of cattle (Limousin and Simmental) became popular in the United States, Canada, Australia and New Zealand. 22/ The desired animals could not be imported because of strict quarantine regulations against foot and mouth disease. However, semen could be shipped into the United States, after a period of quarantine, from specific bulls that were under strict health restrictions. By using artificial insemination, the semen was then used to establish the desired European breed in the United States. A cow of any breed could be inseminated with semen from a bull of a desired foreign breed, for example, Simmental, and the resulting offspring would be one-half Simmental. A one-half Simmental heifer inseminated with purebred Simmental semen would produce a three-fourths Simmental offspring and its

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20/ Sorensen, A. M., Jr. Animal Reproduction: Principles and Practices. McGraw-Hill, 1979. p. 344.

21/ Ibid.

22/ Seidel, George E., Jr., and Sarah M. Seidel. The Embryo Transfer Industry. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, Inc., 1981. p. 42.

mating to a purebred Simmental would result in a seven-eighths Simmental offspring. Most breed associations recognize three-fourths or seven-eighths animals as acceptable for breed recognition. Therefore, it would take three to four generations to produce "purebred" cattle by the above method, or about 5 to 7 years if everything went well, since gestation is about 9 months and a heifer should be at least 12 months old before breeding. While embryo transfer could not decrease the generation intervals, it could be used, along with superovulation, to produce multiple offspring from the desired three-fourths and seven-eighths "purebred" females. Cattle breeders provided the necessary economic incentives which influenced veterinarians and animal scientists into developing embryo transfer as a breeding tool.

The commercial application of embryo transfer is largely confined to the cattle industry. This section will primarily focus on the use of embryo transfer in cattle. The successful development of embryo transfer as an "industry" depended upon the concurrent development of several support technologies, including superovulation, embryo culture, cryopreservation of embryos, estrus detection and estrus synchronization. Each support technology will be discussed in the following subsections.

Superovulation

Embryo transfer programs are dependent upon the production of multiple ova. Since the cow usually only produces single or double ovulations, it must be stimulated to superovulate by injecting the animal with hormones such as pregnant mare's serum gonadotropin (PMSG), follicle stimulating hormone (FSH), human chorionic gonadotropin (hCG) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The exact mechanism of action for these hormone treatments is not understood at the present time.

About midway through the cow's estrous cycle, the animal is injected with PMSG, which causes the ovarian follicles to grow. 23/ Within each follicle is a developing egg, or ovum. Upon maturation of the follicle, it ruptures, releasing the ovum, which will then enter the oviduct. About 2 to 3 days after the PMSG treatment, the animal is injected with $PGF_{2\alpha}$ causing the premature lysis (disintegration) of the corpus luteum. The corpus luteum is the small mass of cells which develops at the site of each ruptured follicle. Estrus occurs about 2 days later and lasts for about 12 hours. Superovulated animals are then artificially inseminated (5 days after the first injection), since superior sires are usually available only through the use of AI. Multiple inseminations are used over a period of several hours since the eggs will not all be released from the ovary at the same time.

Superovulation protocols have been developed for several laboratory and farm animal species; each treatment series of hormone injections varies slightly, depending upon the species. According to George Seidel of the Animal Reproduction Laboratory of Colorado State University, "superovulation increases the number of normal ova recovered by about a factor of 10 for cattle, sheep, and goats but only by a factor of 2 or 3 for swine." 24/ Superovulation with artificial insemination in cattle usually results in 6 to 7 normal embryos and 2 to 3 abnormal embryos per cow. Three to four pregnancies after transfer will result. In 60 to 70 percent of cases of embryo collection without superovulation an ovum or embryo is recovered, 10 to 20 percent of which are unfertilized ova or morphologically abnormal embryos. "Thus, a normal embryo

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23/ Seidel, George E., Jr. Superovulation and Embryo Transfer in Cattle. Science, v. 211, Jan. 23, 1981. p. 351.

24/ Seidel and Seidel, The Embryo Transfer Industry, p. 51.

is obtained 50 percent of the time. Pregnancy rates after transfer of such embryos are close to 70 percent." 25/

When cows are superovulated, variations in response to the hormone injections occur between different breeds of cattle, among different individuals of the same breed and between the first treatment and subsequent treatments of the same individual. 26/ Seasonal effects have also been demonstrated. 27/ Since superovulation results in greatly altered internal hormone levels for the animal, an interval of one to two complete estrous cycles between treatments is usually allowed to occur in order to avoid possible permanent alteration of the animal's natural cycle. 28/

The degree of success with superovulation cannot be predicted accurately for any individual animal; this is the greatest drawback of the technique. 29/ Preparations of PMSG and other hormones vary from batch to batch and are sometimes difficult to obtain. The FDA has recently declared PMSG a new animal drug; its use in the United States requires compliance with FDA regulations.

Embryo Collection

Following superovulation and artificial insemination, embryos are removed from the cow by using either surgical or nonsurgical methods. Surgury is used

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25/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 354.

26/ Betteridge, K. J. Techniques and Results in Cattle: Superovulation. In Betteridge, K. J., ed. Embryo Transfer in Farm Animals: A Review of Techniques and Applications. Canadian Department of Agriculture, Monograph 16, 1977. p. 1.

27/ Ibid.

28/ Seidel and Seidel, The Embryo Transfer Industry, p. 51.

29/ Ibid., p. 52.

to recover embryos in the oviduct, and is the most efficient method for sheep, goats and pigs. Collection rates are higher and less scar tissue is formed when a mid-ventral (abdominal) incision is used rather than a flank incision. After locating the oviducts and uterus, the eggs are flushed from the animal's reproductive system into a collecting dish.

In the non-surgical method, a three-way Foley catheter is inserted through the cervix and into one of the two horns in the cow's uterus. 30/ One channel of the three-tubed catheter inflates a small balloon which prevents the embryo flushing solution from leaving the uterine horn other than by way of the catheter. The remaining two channels of the catheter are for inflow and outflow of the flushing solution. Approximately 500 milliliters of fluid is used to flush each uterine horn. The flushing solution is allowed to drain from the catheter into a collecting dish. The embryos are then examined for normal development. Unfertilized eggs are discarded, along with abnormally developed embryos. "The most consistently useful sign of the embryo's chances of establishing a pregnancy was its stage of development; the less advanced the embryo, the less its chances of surviving." 31/ The average superovulated female produces about ten eggs. Seven of the ten are usually healthy enough to transfer, producing about four calves. 32/ 33/

30/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 352.

31/ Betteridge, K. J. Methods of Assessing the Viability of Embryos. In Betteridge, K. J., ed. Embryo Transfer in Farm Animals: A Review of Techniques and Applications. Canadian Department of Agriculture, Monograph 16, 1977. p. 24.

32/ Caras, Breeding A Revolution, p. 92.

33/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 354.

Embryo Culture/Storage/Cryopreservation

It is sometimes necessary to store embryos for several hours or several weeks or even months before transferring them to recipient females. For short-term storage of less than 24 hours, the embryos are kept at 37 degrees C in a pH buffered salt solution. 34/ For periods longer than 24 hours, the temperature is lowered, and the culture media is usually more complex, containing carbohydrates, amino acids, vitamins and nucleic acid precursors. Bovine serum albumin or heat-inactivated blood serum may also be added. 35/ "Pregnancy rates for embryos cultured up to 24 hours are similar to those of embryos cultured for only a few hours, but there may be more subsequent embryonic death after transfer of embryos cultured for 24 hours or more. For commercial purposes, embryos are usually kept in vitro for less than 10 hours." 36/ "Embryos from cattle, sheep and goats tolerate cooling to 0 degrees to 10 degrees C for several days without substantial loss of viability, but pig embryos do not survive cooling below 15 degrees C." 37/ Embryos may also be stored in the oviducts of living rabbits for 2 to 4 days. They continue to develop normally and have high pregnancy rates after they are transferred to the recipient animal.

For longer periods of time, embryos may be stored indefinitely at -196 degrees C in liquid nitrogen. Cryopreservation or deep-freezing of embryos destroys about one-third of the embryos, "but pregnancy rates of the

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34/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 352-353.

35/ Seidel and Seidel, The Embryo Transfer Industry, p. 56.

36/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 353.

37/ Seidel and Seidel, The Embryo Transfer Industry, p. 55.

other two-thirds are not much lower than those of fresh embryos." 38/ Freezing is sometimes used commercially if embryo recipients are not available.

Estrus Detection/Synchronization

Before an embryo transfer can occur the recipient cow must be in the same stage of the estrous cycle as the embryo donor. If the recipient's cycle is off by more than one day, there is much less chance of a pregnancy occurring. The largest cost of embryo transfer is arranging for the needed number of recipient females and maintaining them in a nonpregnant state. This expense is the major reason why embryo transfer is so much more costly than artificial insemination. 39/

Recipients are made available either by having a large enough herd of females, all at different stages of estrus, or by using injections of $PGF_2\alpha$ or progestogens. There is little difference in pregnancy rates between the two methods. The advantage of the hormone injection method is that it allows for the use of a smaller herd of recipient animals. However, if large numbers of embryo transfers are routinely made, then there is little or no cost advantage to the hormone injection method. In addition, the animals must be given the hormone treatments before the required number of recipients needed for the embryo transfers is known. (Therefore, an advantage to embryo freezing is that the embryos would wait for the recipients instead of vice versa. 40/) "The importance ascribed to the technology of estrus synchronization is best illustrated by the fact that more research effort, in terms of man-years or dollars, has been devoted to this problem by universities, Government agencies

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38/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 353.

39/ Ibid.

40/ Ibid.

and private industry over the last decade then any other technology related to reproduction." 41/

No matter which method of estrus synchronization is used, estrus detection is still necessary. Animals are observed two to three times daily for signs of estrus, which include allowing other animals to mount, excessive mucus secretion, nervousness and increased walking. 42/ Various marking devices may be attached to bulls that are physically incapable of breeding. The devices leave paint or grease marks on the backs of cows who allow themselves to be mounted. 43/

Embryo Transfer

Both surgical and non-surgical methods of embryo transfer have been developed. Surgical transfer is required for embryos in the very early stages of development. Surgery is also the only method of embryo transfer in sheep, goats and pigs. 44/ Approximately 50 percent of commercial embryo transfers in cows are surgical. 45/ There are two approaches to the surgical transfer:

1. The mid-ventral incision, which requires general anesthesia, but may be less traumatic to the reproductive system and permits better exposure in some animals; or
2. The flank incision, with local anesthesia, which is faster, less expensive, but has a slightly lower pregnancy rate.

After exposing the reproductive system using a mid-ventral incision, the presence of a corpus luteum on an ovary is determined. The corpus luteum is necessary for

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41/ Seidel and Seidel, The Embryo Transfer Industry, p. 55.

42/ Sorensen, Animal Reproduction, p. 235-237.

43/ Ibid., p. 298-304.

44/ Seidel and Seidel, The Embryo Transfer Industry, p. 57.

45/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 353.

the maintenance of pregnancy after the transfer has been performed. 46/ The embryo is then transferred to the uterine horn which is on the same side as the corpus luteum, where survival rates are about three times higher than in the other horn. 47/ A small incision is made through the uterine wall and a glass pipette is passed into the uterus. The embryo is expelled into the uterus by means of a syringe attached to the pipette.

In the flank approach, the presence of an active corpus luteum is determined by rectal palpation of the ovaries. 48/ The flank is surgically prepared and a 15-centimeter incision is made. The horn of the uterus is pulled into the incision and the embryo is inserted as described above. There is an increased risk of infection with this method because of the movements of the cow during surgery. In addition, the optimal site for depositing the egg is not always accessible. However, it is used more frequently by commercial embryo transfer units because it saves time and can be done in the field with a minimum of equipment. 49/

The non-surgical method uses equipment similar to that used for artificial insemination. 50/ The presence of a corpus luteum is determined by rectal examination of the ovaries. The transfer equipment is inserted through the cervix into the appropriate uterine horn and the embryo is expelled into the lumen of the uterus. Care must be taken to prevent infection even with the non-surgical method since the "progesterone-dominated endocrine environment

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46/ Sorensen, Animal Reproduction, p. 350.

47/ Elsden, R. P. Embryo Transfer by Surgical Methods. In Betteridge, K. J. Embryo Transfer in Farm Animals: A Review of Techniques and Applications. Canadian Department of Agriculture, Monograph 16, 1977. p. 27.

48/ Ibid.

49/ Seidel and Seidel, The Embryo Transfer Industry, p. 57.

50/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 353.

makes the uterus especially prone to infection. In contrast, it is extremely difficult to infect the uterus when cows are routinely inseminated because of estrogen domination at that stage of the estrous cycle." 51/

Possible reasons for the failure of embryo transfers are:

1. loss of the embryo because it adhered to the transfer equipment or was not deposited in the appropriate place in the uterus;

2. excessive trauma to the reproductive tract;

3. infertility of the recipient--"often . . . the only animals available as recipients are ones culled from breeding herds for failure to become pregnant." 52/

Applications of Embryo Transfer

The following seven applications of embryo transfer are currently feasible. 53/

1. Obtain more offspring from valuable females. Superovulation and embryo transfer can be used to increase the number of calves produced by a superior cow.

> In dairy cattle, for example, 70-90 percent of female calves must be reared as replacements for animals culled for reasons other than low production. If the replacements were selected from the top 10 percent of the herd by amplifying the reproduction of superior cows by embryo transfer, the rate of genetic gain from the female side could be increased three to four times. The National Cooperative Dairy Herd Improvement Program reported that the 275 embryo transfer donors on record were at the 75th percentile for genetic value for milk production, whereas their 50 progeny by embryo transfer that were old enough to have records were at the 87th percentile. The increased genetic value over the population average that these cows would transmit to offspring was estimated to be 112 kg of milk for the donors and 155 kg for their embryo transfer offspring. 54/

51/ Ibid., p. 353.

52/ Seidel and Seidel, The Embryo Transfer Industry, p. 62.

53/ Ibid., p. 63-65.

54/ Ibid., p. 63-64.

2. Obtain offspring from infertile females. Infertility due to age or disease can be circumvented using embryo transfer, since "the uterus often loses the ability to support a pregnancy before the ovary ceases to produce ova." 55/

3. Export and import of livestock. Because of their smaller size, embryos are obviously easier and less expensive to transport than adult animals. In addition, there is less chance of introducing new diseases to the importing area when embryos are used rather than adult animals. Adaptation to the new environment is easier for an embryo than for an adult, since the female recipient of the embryo will impart to it passive immunity to the local diseases.

4. Test for undesirable genetic traits. Superovulation and embryo transfer enable the livestock breeder to determine if an animal carries undesirable genetic traits in a shorter period of time than was previously possible. This is because the two techniques provide the opportunity to test many more offspring than would be possible with natural reproduction.

5. Introduce new genes into pathogen-free herds. The use of "total-confinement units" for animal breeding is becoming more common in the swine industry chiefly because of high labor costs and increased land value. This method also allows for closer supervision of the pig's diet and more effective control of diseases and parasites. 56/ However, the introduction of new adult animals from other areas for breeding purposes can potentially expose the confinement-bred animals to diseases for which they do not have immunity. Embryo transfer greatly reduces the risk of disease transmission.

55/ Ibid., p. 64.

56/ Pond, Wilson G. Modern Pork Production. Scientific American. May 1983. p. 96.

6. Increase the population base of rare or endangered animals. The embryos of rare species can be transferred to similar, but more plentiful species; for example, American bison embryos may be transferred to domestic cattle. 57/ Embryo transfer could also be of great benefit to zoos or animal sanctuaries in their efforts to maintain or increase the numbers of captive endangered animals. Animals in different zoos could be bred, "thus increasing breed choices when costs, quarantine problems or trauma make shipping of adults impossible." 58/

7. Research. Embryo transfer is a technique for use in research involving in vitro studies of gametes or embryos.

Economic Impact of Embryo Transfer

Approximately 17,000 bovine embryo transfer pregnancies occurred in 1979, costing breeders about $20,000,000. 59/ "Financial volume for 1979 was estimated as 9,000 pregnancies at $1,800 each plus 8,000 'in house' or 'on farm' pregnancies at $500 each. This probably is an under estimate. Costs per pregnancy will vary depending on which costs are ascribed to embryo transfer." 60/

Charges for embryo transfer in cattle can vary from $300 to $2,000 per pregnancy; costs for horses are higher, and sheep, goats and swine are considerably lower. 61/

57/ Seidel and Seidel, The Embryo Transfer Industry, p. 65.

58/ Ibid.

59/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 351.

60/ Ibid., p. 357, footnote 11.

61/ Seidel and Seidel, The Embryo Transfer Industry, p. 44.

> In addition, there are many indirect costs, such as registration and blood typing fees, advertising, or loss of production by the donor while she is under treatment. When these costs are added up and adjusted for the post-partum salvage value of the recipient and the current net interest cost of money, it is economically justifiable for a breeder in the United States to use embryo transfer if he can obtain an average of about $2,500 per calf at six months of age, including both bull and heifer calves. . . . Some embryo transfer offspring command high prices. For example, the top-selling animal in a recent National Holstein Convention Sale sold for $131,000, and the topselling heifer in the 1980 ABS Americana sale sold for $22,000. 62/

However, the current high costs of embryo transfer cannot be recovered in increased meat and milk production. 63/

> From the breeder's point of view, the success of embryo transfer is variable, depending not only on the number of pregnancies, but also on market fluctuations, promotional opportunities, management techniques and the breeder's goals. Nevertheless, breeder's comments and the steady increase in use of embryo transfer services attest to the fact that cattle breeders believe embryo transfer is profitable, both for the improvement of their herds and for sales of breeding stock. 64/

C. SEXING OF SPERM AND EMBRYOS

The sex of a mammal is determined at conception and depends upon whether an egg bearing an X chromosome is fertilized by an X chromosome-bearing sperm or a Y chromosome-bearing sperm. Embryos with two X chromosomes will develop into females; those with an X and a Y chromosome will develop into males. Thus, if X-bearing and Y-bearing sperm could be separated, sex selection prior to fertilization would be possible via artificial insemination.

62/ Ibid., p. 44-45.

63/ Ibid., p. 49.

64/ Ibid.

Several approaches to sperm separation have been attempted, including methods based on differences in mass, electrical charge and motility. 65/ Another way to predetermine sex would be differential inactivation of either X- or Y-bearing sperm. Methods of inactivation have been tried include treating semen with hormones, chemicals, altered atmospheric pressure, or antibodies against Y-bearing sperm. 66/ Researchers have also tried immunizing the female against sperm bearing the Y chromosome. Unfortunately, at the present time no method of sperm separation or inactivation has been successfully developed. 67/

Another approach to sex selection is embryo sexing, or diagnosing the individual's sex after fertilization. Presumably, after the embryos resulting from a superovulation have been sorted by sex, those of the desired sex would be transferred via the methods previously discussed into recipient females. Genetic Engineering, Inc. (Denver, Colorado) claims to have developed such an approach. 68/ Since male embryos carry on their cell surfaces a specific protein antigen, Genetic Engineering, Inc. developed a method of determining the presence of this antigen. Embryos carrying the antigen are males, those that do not are females. The company uses antibodies tagged with a fluorescent die marker to detect the male-specific antigen.

65/ Betteridge, Keith J., W. C. D. Hare and Elizabeth L. Singh. Approaches to Sex Selection in Farm Animals. In Brackett, Benjamin G., George E. Seidel, Jr. and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 111.

66/ Ibid.

67/ Ibid., p. 111-112.

68/ Embryo Sexing: Vive la Difference. Economics, v. 286, Feb., 19, 1983, p. 103.

> In mice, this approach works with an 80 percent reliability. But cattle embryos present a problem. The difficulty is that the number of antigen targets on the surface of an embryo varies over time. When the embryo comprises only eight cells--about three days after conception--a large number of antigens are expressed on its surface. Unfortunately, by the time that it can be removed from the cow--about six days after conception--this is no longer so. 69/

For these reasons, the embryo sexing process developed by Genetic Engineering, Inc. is viewed with some scepticism by other recombinant DNA companies and academic researchers.

D. IN-VITRO FERTILIZATION

The technology of *in vitro* fertilization allows an egg to be fertilized by a sperm outside the body, literally, "in glass". Although *in vitro* fertilization of ova has been attempted in several mammalian species, including pigs, sheep, and cows, "it has been possible to circumvent the oviduct (the natural site of fertilization) with *in vitro* fertilization and embryo transfer only in mouse and man." 70/

Infertility in mammals "may reflect disease conditions (1) that result in defect egg production, (2) that prevent fertilization of a normal egg or (3) that prevent development of the embryo following fertilization." 71/ In the second and third instances, *in vitro* fertilization and embryo transfer could be used to alleviate the animal's infertility. These two techniques could also be used when fertilization problems are attributed to the male, for example, because of

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69/ Ibid.

70/ Brackett, Benjamin G. Applications of *In Vitro* Fertilization. In: Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 142.

71/ Ibid., p. 156.

low sperm count in semen caused by non-genetic factors. "In a case study of repeat breeder cows, 40.8 percent fertilization failure was observed. If viable ova could be recovered from such cows, in vitro fertilization should provide the means for overcoming problems of sperm-egg meeting." 72/

E. PARTHENOGENESIS, CHIMERAS, TWINNING AND CLONING

Parthenogenesis is defined as "reproduction without the genetic participation of the sperm." 73/ The fertilization of an egg does not take place. Parthenogenesis occurs commonly in nature, particularly among invertebrates (animals without internal backbones). It occurs naturally in some populations of reptiles, amphibians, and fish. 74/ Although birds are capable of parthenogenesis, it rarely occurs in these animals. Mechanisms of parthenogenesis probably vary from one species to another.

Chimeras are individuals produced by combining parts of one embryo with parts of another. The embryos may be of the same or a different species. The term can also be used to refer to a person who has received a transplant of genetically and immunologically different tissue, such as bone marrow. 75/

Twinning is the production of equivalent structures by division. 76/ Identical twins are formed in this way; they develop from a single fertilized

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72/ Ibid.

73/ Markert, Clement L., and George E. Seidel, Jr. Parthenogenesis, Identical Twins, and Cloning in Mammals. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 193.

74/ Ibid.

75/ Stedman's Medical Dictionary, 22d Edition. Baltimore, The Williams & Wilkins Company, 1972. p. 237.

76/ Ibid., p. 1346.

egg that very early in development divides into two independently growing cell aggregates.

A clone is a group of organisms which have arisen from a single individual as a result of asexual reproduction. 77/

Parthenogenesis, the formation of chimeras, twinning and cloning, are all asexual ways of creating new individuals. They are of interest to geneticists and developmental biologists who wish to learn more about how the information stored in genes is expressed and how organisms develop from eggs to adults. They are also of interest to livestock breeders who like to produce exact copies of outstanding domestic animals. 78/

Chimeras are used in experiments to answer questions about mammalian development. Mice are commonly used for chimera studies. Thousands of mouse chimeras have been manufactured in a process that begins by flushing 4- to 8-cell embryos from female animals. Once the outer coating (called the zona pellucida) is removed, the embryos' cells are mixed together, cultured for a period of time, then implanted into recipient females. 79/ In 1978, scientists at Yale University succeeded in making chimeras derived not from the usual two aggregated mouse embryos, but from three, thus forming individuals with six genetic parents. 80/

Information derived from studies of organisms formed by parthenogenesis may help scientists to answer fundamental questions about cloning. Parthenogenesis, like cloning, is a method of "selfing." It produces offspring fairly similar

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77/ Ibid., p. 260.

78/ Markert and Seidel, Parthenogenesis, Identical Twins, and Cloning in Mammals, p. 182.

79/ Markert, Clement L., and Robert M. Petters. Manufactured Hexaparental Mice Show That Adults are Derived from Three Embryonic Cells. Science, v. 202, Oct. 6, 1978. p. 56.

80/ Ibid.

to, but not genetically identical with, the mother. A major obstacle of both procedures is the activation of development. In parthenogenesis the egg must be activated without sperm. Cells must also be activated in some methods of cloning. 81/ Since mammals fail to develop to term via parthenogenesis, scientists reason that there may be unknown biological barriers to successful parthenogenetic development. Such barriers would probably have profound consequences for cloning mammals. 82/

Researchers at Colorado State University have been working on a type of cloning in cattle that is very much like natural twinning. Over the last year and a half, 600 such experiments have been performed. 83/ Embryos are flushed from the natural mother about seven days after fertilization. Under a microscope set at a magnification of 200 times, each embryo is cut in half. The two halves are implanted in two recipient cows whose estrous cycles are synchronized with the natural mother's cycle. The procedure has successfully produced two sets of identical twin calves. The two heifers and two bulls are the first registered beef calves to be cloned and born on an American farm. 84/ The cattle twinning procedure may allow breeders to produce far more high quality animals than would be possible otherwise.

81/ Markert and Seidel, Parthenogenesis, Identical Twins and Cloning in Mammals, p. 182.

82/ Ibid.

83/ Orr, Richard. Calf Cloning is Breeding Milestone. Chicago Tribune, May 10, 1983. p. A3.

84/ Ibid.

F. NUCLEAR TRANSPLANTATION

Amphibians

Nuclear transplantation, (also called cloning by some investigators) was first developed in amphibians (frogs and toads) to test a fundamental question of developmental genetics: do differentiated cells (those that have assumed their mature form and function) retain the full genetic potential of a fertilized egg? Successful nuclear transplantation requires the insertion of a totipotent nucleus (one capable of directing the cell to develop into an entire organism) into a mature egg from which the original nucleus has been removed.

Such experiments in the laboratory of Dr. J. N. Gurdon showed that normal toads can develop from fertilized eggs containing nuclei transplanted from developing (embryonic or tadpole) brain, liver, and intestinal cells. 85/ However, further experiments with mature cells indicate that at some point in development, a cell's DNA becomes irreversibly modified so that the full complement of genes can no longer be expressed. The mature cells' nuclei failed to allow the fertilized eggs to develop. 86/ Although the nuclear transplantation procedure can be described simply, it is actually quite difficult and requires an unusually high level of manual dexterity and microsurgical skill. 87/

85/ Goodenough, Ursula and Robert P. Levine. Genetics. New York, Holt Rinehart and Winston, Inc., 1974. p. 717.

86/ McKinnell, Robert G. Amphibian Nuclear Transplantation: State of the Art. In Brackett, Benjamin G., George E. Seidel and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 165.

87/ Ibid.

Mammals

Nuclear transplantation in mammals is similar to previously described experiments with amphibians. The necessary requirements include a source of eggs, a supply of nuclei from embryonic cells, a procedure for removing nuclei, and a technique for inserting the nuclei into eggs. The surgically altered egg must be cultured in vitro until it can be transferred to an appropriate host, and the uterus that receives the cultured embryo must be in the proper phase of the estrous cycle. 88/

In 1981, Karl Illmensee of the University of Geneva, Switzerland and Peter Hoppe of the Jackson Laboratory, Bar Harbor, Maine, reported the successful "cloning" of three mice. The mice were produced by the transplantation of nuclei from 3- to 4-day-old embryos into recently fertilized eggs whose own nuclei had been removed. 89/ Newspaper reports that spread in the wake of Illmensee and Hoppe's research article were called misleading by critics who objected to the use of the term "cloning" to describe what they saw as nuclear transplant manipulations. The work, these critics claimed, fell short of actual cloning which they asserted would involve producing a mature mouse from a single cell of another mature mouse. 90/

Controversy still surrounds the Illmensee and Hoppe experiments. A committee at the University of Geneva is investigating other experiments performed by Illmensee for "alleged irregularities in the reporting of research data. . . .

88/ Ibid., p. 176.

89/ Marx, Jean L. Three Mice "Cloned" in Switzerland. Science, v. 211, Jan. 23, 1981. p. 375.

90/ Misleading Cloning Reports Circulated. Chemical & Engineering News, Jan. 12, 1981. p. 7.

The irregularities . . . involve alterations made in experimental protocols after the experiments were completed." 91/ The experiments under investigation were performed in 1982 and "involve the transplantation of nuclei from cancer cells into fertilized eggs whose own nuclei had been removed." 92/ The experiments under investigation are similar in design to those performed in 1981 with Hoppe in Bar Harbor and described above. According to the vice-rector of the University of Geneva, Illmensee has requested that the committee also review his other work, "which has now come under a cloud." 93/ Attempts to reproduce the 1981 Illmensee and Hoppe experiments in mice "have proved difficult, according to Clement Market, an embryologist at Yale University." 94/ A committee at the Jackson Laboratory recently completed its investigation into the work performed by Illmensee in 1981 while he was a visiting professor at the Bar Harbor, Maine facility. The committee's report indicated that it found no evidence of fraud, but recommended that Hoppe and Illmensee repeat their 1981 experiments since the work has not been able to to be verified by other investigators. 95/

Applications

The value of nuclear transplantation, according to many researchers, lies in what the experiments can tell scientists about normal and abnormal development;

91/ Marx, Jean L. Swiss Research Questioned. Science, v. 220, June 3, 1983. p. 1023.

92/ Ibid.

93/ Ibid.

94/ Ibid.

95/ Marx, Jean L. Bar Harbor Investigation Reveals No Fraud. Science, v. 220, June 17, 1983. p. 1254.

it was never designed to be used as a reproductive procedure for domestic animals. 96/ Nevertheless, scientists and livestock breeders are interested in its potential application to livestock reproduction. 97/ Conceivably, producing many copies of a valuable pedigreed animal by transplanting nuclei from its body cells into enucleated eggs would be a valuable tool for livestock breeders. However, nuclear transplantation experiments in mammals have all utilized nuclei from embryo cells and not from adult cells. This means that the exact genetic make-up, or characteristics, of the animal formed from such an experiment is unknown in contrast with an experiment using adult cell nuclei. Despite the opinions of many scientists that transplantation experiments with adult cell nuclei may never succeed, researchers are still intrigued with the theory of cloning. Research in the field will undoubtedly continue. 98/

G. GENE TRANSFER

The transfer of genes from one organism to another takes place each time an egg is fertilized. The process allows new and unique combinations of genetic material to occur, and is probably an important reason for the evolutionary success of sexual reproduction. 99/

Even with careful breeding programs, the transfer of specific genes cannot be strictly controlled. In efforts to learn more about how genes work, scientists

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96/ Marx, Jean L. More about Cloned Mice. Science, v. 212, Apr. 10, 1981. p. 145.

97/ McKinnell, Amphibian Nuclear Transplantation, p. 164.

98/ Markert and Seidel, Parthenogenesis, Identical Twins and Cloning in Mammals, p. 196.

99/ Solter, Davor. Gene Transfer in Mammalian Cells. In Brackett, Benjamin G., George E. Seidel and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 201.

have developed methods to control the transfer of selected genes between animals. Recombinant DNA techniques allow scientists to insert a particular gene into a given cell or whole organism and to study how that gene is controlled. 100/

Methods

Two procedures have been developed for transferring specific genes into animal cells. In the first method the desired gene is linked to the DNA of an animal virus. The animal cells are then infected with the virus, which is carrying the new gene. Using this method, scientists have linked a rabbit protein gene (beta-globin, a component of the oxygen carrying molecule hemoglobin) with the DNA of SV40, a monkey virus. Monkey cells were then infected with the recombinant SV40 virus, which enabled the cells to make the rabbit protein. 101/

In the second method, DNA containing the desired gene is identified, isolated and then mixed with animal cells. A small percentage of these cells will internalize the DNA and make use of the new gene. Using this method, scientists at Columbia University College of Physicians and Surgeons were able to correct a genetic defect in mouse cells that lacked the enzyme thymidine kinase (TK). The scientists had in effect actually "cured" the cells of their genetic defect. In another experiment at the University of California, Los Angeles (UCLA), scientists used this method on cells which were subsequently implanted into living animals. In 1980, a team of UCLA scientists inserted the gene for an enzyme, called dihydrofolate reductase, into mouse bone marrow cells. The cells were

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100/ Ibid., p. 201-202.

101/ Anderson, W. French and Elaine G. Diacumakos. Genetic Engineering of Mammalian Cells. Scientific American, v. 245, July 1981. p. 106.

then implanted into mice, where they made the enzyme according to the transferred gene's instructions. 102/

The only way a specific gene can be incorporated into every cell of an animal is by introducing the gene into fertilized eggs or very young embryos. 103/ The first successful experiment of this kind was reported in 1977 by Dr. Rudolf Jaenish. 104/ Dr. Jaenish and his associates infected 4-16 cell stage embryos with a virus and then implanted the embryos into the uteri of recipient females. Some of the experimental embryos developed into adults whose reproductive cells had incorporated the viral genetic material. When these adults were mated, the viral genome was transmitted to all their progeny. 105/

In another method, the gene to be transferred is directly injected, with a tiny glass micropipette, into the fertilized egg. Sometimes the DNA is injected into the pronucleus of the fertilized egg. The pronucleus is a sperm that has penetrated the egg cell. At this point in development, the egg naturally accepts new DNA, so it is a logical stage in which to inject foreign genes. However, not all of the adult animals which develop from injected eggs make use of (or express) the transferred gene. Of those that do, not all produce offspring containing the gene. This indicates that only some of the adult animal's cells that developed

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102/ Cline, M. J., et al. Gene Transfer in Intact Animals. Nature, v. 284, Apr. 3, 1980. p. 422.

103/ Solter, Gene Transfer in Mammalian Cells, p. 214.

104/ Ibid.

105/ Ibid.

from the injected egg contain the new gene. 106/ Scientists continue to refine these procedures.

Applications

For the most part, scientists used gene transfer to answer fundamental questions about gene control, function, and expression. But as techniques of transferring selected genes between animals of the same or even different species improve, scientists are beginning to speculate about their possible application to animal husbandry. For example, gene transfer could be used to improve valuable livestock traits, such as fast growth or high milk production.

Scientists at the University of Washington, the University of Pennsylvania and the Salk Institute recently succeeded in transferring the gene for rat growth hormone into mice. The resulting mice were twice as big as animals that did not receive the foreign gene. The increased size of the mice was not because the growth hormone gene came from a rat, but because the scientists had attached to the rat gene a second gene which they used to control the level of expression of the rat growth hormone gene. The metal zinc was included in the animal's diet. The zinc activated the second gene, which in turn increased the amount of rat growth hormone made by the rat gene. 107/ 108/

106/ Marx, Jean L. Still More about Gene Transfer. Science, v. 218, Oct. 29, 1982. p. 459.

107/ Williams, Jeffrey G. Mouse and Supermouse. Nature, v. 300, Dec. 16, 1982. p. 575.

108/ Marx, Jean L. Building Bigger Mice through Gene Transfer. Science, v. 218, Dec. 24, 1982. p. 1298.

II. HEALTH TECHNOLOGIES

A. VACCINE PRODUCTION

The term vaccine is derived from the Latin word vaccinus which means "of or from cows." 109/ The first vaccine was made about 200 years ago by Edward Jenner, who injected live cowpox virus into a young boy's arm to protect him against smallpox. Since Jenner's time, the term vaccine has come to encompass any preparation used to stimulate the recipient's immune system to produce protective antibodies against a pathogen (disease-causing organism). These preparations may include killed bacteria; attenuated (weakened) but live viruses; or derivatives of microbes, fungi; and parasites. 110/ The use of the term vaccine is sometimes confined to viral preparations and the term bacterin is used for preparations made with bacteria.

All vaccines work in essentially the same manner: they stimulate an immune response against the invading organism. When a vaccine enters the body, certain protein molecules, or antigens, on the microorganisms contained in the vaccine are recognized as "foreigners" by the body's surveillance cells. These cells trigger other cells, called lymphocytes, to produce antibodies against the foreign antigens. Without actually producing symptoms of the disease itself, a vaccine sensitizes the lymphocytes so that they can produce the same antibodies rapidly when exposed to the corresponding disease-causing strain of viruses or bacteria.

109/ Webster's Seventh New Collegiate Dictionary. Springfield, G. & C. Merriam Co., 1965. p. 979.

110/ Stedman's Medical Dictionary, 22d Edition. Baltimore, The Williams & Wilkins Co., 1972. p. 1366.

Producing the large quantities of pathogens necessary for vaccine manufacture can be difficult and expensive. Despite the fact that virulent viruses and bacteria can evade the body's protective mechanisms and multiply in their hosts, they are often quite difficult to grow in culture outside the body. Sometimes they are even difficult to grow in laboratory animals.

There are two forms of vaccine currently being used by the medical community: attenuated (weakened) live organisms and inactivated (dead) organisms. Using attenuated, live organisms is the preferred method of vaccination because it is more likely to induce long-lasting protection. Attenuated virus vaccines may also be referred to as modified live virus vaccines.

The attenuation process alters the organism so that it will no longer cause disease symptoms. However, the growth characteristics and the surface antigens of the organism must not be changed in the attenuation process. If the organism cannot grow, it cannot infect, colonize, and immunologically sensitize the vaccine recipient. If the antigens are changed, the attenuated organism will not trigger production of the appropriate antibodies, and therefore it would be useless as a vaccine. But attenuated virus vaccines are risky since the microbes can revert, through a genetic change other than the one that weakened them, to a virulent form and actually produce the disease they were meant to protect against. 111/

Vaccines containing killed (inactivated) microbes are generally considered safer than those containing attenuated organisms, but the inactivation process often reduces the ability of the preparation to induce immunity. In addition,

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111/ President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Splicing Life. Washington, U.S. Govt. Print. Off., 1982. p. 37.

the inactivation process which uses formalin or formaldehyde can cause unwanted side effects, such as allergic reactions, in recipients. There is also the danger that at least one pathogenic organism has survived the inactivation process and will thereby cause the disease in those individuals who receive the contaminated vaccine. 112/

A further problem lies in the instability of vaccines made from either killed or attenuated organisms. These vaccines must be kept under refrigeration--a necessity that limits their usefulness in Third World countries where they are most urgently needed. 113/

So in spite of the advances in health care which have been accomplished by using traditional vaccines, there is still room for improvement. New and improved vaccines can be produced by using the recently developed and continually evolving technology called genetic engineering.

The term "genetic engineering" was coined in 1965 to describe "...what has become to be a wide range of techniques by which scientists can add genetically determined characteristics to cells that would not otherwise have possessed them." 114/ Of particular importance to vaccine production is the genetic engineering technique known popularly as gene splicing. This technique, also referred to as recombinant DNA, was made possible by the discovery, in the early 1970s, of restriction enzymes, proteins that exist in nature as part of the defense system bacteria use to respond to foreign DNA. 115/ DNA (deoxyribonucleic acid) is the molecular basis of heredity for most organisms. 116/

112/ The New Vaccines, p. 43.

113/ The New Vaccines, p. 43.

114/ President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Splicing Life, p. 8.

115/ Ibid., p. 32.

116/ Webster's New Collegiate Dictionary, 1974. p. 335.

Restriction enzymes, of which about 150 have been identified so far, cut DNA at points precisely determined by the chemical sequence of the molecule. The cut segments may contain several genes or only a part of a gene, (the basic functional unit of inheritance) depending on the spacing of sites recognized by the restriction enzymes.

The segment of DNA removed from a chromosome (a string of genes, composed of DNA and protein) can be inserted into a completely different cell by first attaching it to either a virus or a ring of DNA known as a plasmid. Viruses and plasmids act as vectors, carrying the newly attached DNA segment into another cell, usually a bacterial cell. Escherichia coli, which is normally normally present in the human intestine, is the bacterium commonly used for recombinant DNA studies.

Once inside the bacterium, the new piece of DNA is replicated along with the microbe's own DNA each time the cell divides. After many such divisions, a clone of identical cells is formed. Each cell contains a faithful copy of the original recombinant DNA insert. 117/ The bacteria then use their own biological machinery to construct the protein product specified by the inserted gene. Scientists can now use recombinant DNA techniques to make vaccines by allowing bacteria to synthesize the necessary antigens required for the body's response to disease. As mentioned earlier, antigens are the part of the disease-causing microbe which stimulate the protective response of the immune system.

The advantages of vaccines produced by recombinant DNA over either attenuated or killed virus vaccines are numerous. The vaccine cannot be contaminated by virulent strains of microorganisms, it is easy to purify, can be produced in unlimited quantities by rapidly growing bacteria, and is very stable.

117/ U.S. Congress. House of Representatives. Genetic Engineering, Human Genetics, and Cell Biology: Evolution of Technological Issues. Washington, U.S. Govt. Print. Off., Aug. 1980. p. 9.

Before recombinant DNA vaccines can be produced, the genes coding for the critical antigens must be isolated. In the case of many disease-causing organisms, this means identifying more than one gene, since there are usually several different strains, each with slightly different antigens. A vaccine effective against pneumonia, for example, would need to contain about 83 different antigens. 118/

An even newer approach to vaccine production uses standard chemical procedures to synthesize small portions of the protein antigen instead of the entire antigen as described above. The segment of a protein antigen that produces the sensitizing response essential to immunity is identified and then used as a vaccine. However, a protein's shape and function is determined by more than just the class of its constituent amino acids and the sequence in which they are linked. A protein's three dimensional structure also is important in determining its action. X-ray crystallography and computer modeling are used to analyze a protein's three-dimensional configuration.

Recently, computer analysis was used to predict the critical portions of the hepatitis B virus surface antigen. Two peptides containing one of these regions have been synthesized. The vaccine prepared from the peptides was injected into laboratory mice. The mice responded as expected--they produced antibodies to the hepatitis B virus. 119/

Synthetic peptide vaccines have some of the same advantages as recombinant DNA vaccines. They avoid using pathogens, so there is no danger of contamination by virulent microbes. Synthetic vaccines are very pure, unlimited quantities can be produced quickly and cheaply, and the preparations don't need to be

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118/ The New Vaccines, p. 43.

119/ Zuckerman, Arie J. Developing Synthetic Vaccines. Nature, v. 295, Jan. 14, 1982. p. 98.

refrigerated. But synthetic peptide vaccines are not very potent. Scientists still need to develop peptide carriers which boost the effectiveness of these vaccines. 120/

Producing both the synthetic peptide and recombinant DNA vaccines depends on isolating the critical surface antigens of disease-causing organisms, a task that was difficult and time consuming until the development of another genetic engineering advance--cell hybridization. This procedure, which produces hybrid cells called hybridomas, allows scientists to make highly specific antibodies that can be used as "probes" for antigens that may be useful in vaccine development.

In this procedure, scientists first stimulate research animals (usually mice) to produce antibodies by injecting them with the pathogenic organism against which they are interested in developing a vaccine. The antibody-making white blood cells are collected from the mice and then fused with malignant cells (called myelomas) through a chemical process that dissolves and then regenerates the cells' outer membranes. The resulting hybridomas inherit qualities from both parent cells--the cancer cell's ability to grow and divide rapidly and indefinitely and the white cell's ability to produce antibodies. Each hybridoma is cultured individually and eventually produces a clone of identical cells, all synthesizing the same antibody. This antibody is termed a monoclonal antibody because of its production by a clone of cells descended from a single hybridoma. 121/

The great advantage of monoclonal antibodies is their specificity for a single type of antigen or even a small part of an antigen. Using special

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120/ The New Vaccines, p. 46.

121/ President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Splicing Life, p. 35.

radioactive labeling procedures, scientists are able to detect the antigen to which a monoclonal antibody reacts. That antigen can then be used in vaccine development.

Since pathogenic organisms evolve and become resistant to what once were reliable treatment methods, the production of effective vaccines becomes increasingly important. Genetic engineering and cell hybridization should help meet the need for new vaccines and treatment methods.

The following sections describe the development of several genetically engineered vaccines for a number of diseases which affect agriculturally important livestock.

Viral Vaccines

Foot and Mouth Diseases

The National Academy of Sciences has identified 20 animal diseases for which vaccines should be developed. Foot and mouth disease (FMD) is near the top of that list. 122/ Indeed, for the last four centuries, FMD has been one of the world's major animal plagues. 123/

Caused by a virus similar to that responsible for polio, foot and mouth disease affects some 32 species of cloven-hoofed animals, including cattle, swine, sheep, and goats. The infection is highly contagious and severely debilitating, but mortality rarely exceeds 6 percent in any species. Infected animals show fever, weakness, loss of appetite, and blisters on the feet, mouth, and tongue. Abortion, mastitis, and chronic deformitites of the feet are common. In dairy

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122/ The New Vaccines, p. 44.

123/ Bittle, James L. et. al. Protection Against Foot-and-Mouth Disease by Immunization with a Chemically Synthesized Peptide Predicted from the Viral Nucleotide Sequence. Nature, v. 298, July 1, 1982. p. 30.

cows, milk production is severly diminished. Animals that survive infection may become carriers of the virus, perpetuating the disease. In rare instances, the virus has even infected people. 124/

At least for now, foot and mouth disease has been eradicated in the United States; the last major outbreak occurred in 1954. 125/ But in many parts of the world the disease is endemic (native), costing farmers tens of thousands of animals and billions of dollars a year. Quarantine of the infected area and slaughter of all infected and susceptible animals in contact with the disease is the method of control in countries where FMD does not normally occur. Some years ago, British authorities had to destroy more than 280,000 animals to contain a major outbreak. Vaccination is used where the disease is endemic. Over 1,000 million doses of vaccine are used each year. 126/

There are several problems with the vaccine that is currently being used. Perhaps most important for disease eradication is the fact that the vaccine cannot offer permanent immunity. Another problem is that the vaccine is unstable and requires refrigeration, which is difficult to provide in some parts of the world where the need for the vaccine is greatest. 127/ Furthermore, foot and mouth disease viruses are very hardy. Some can escape inactivation, creating a vaccine that causes the very disease it was intended to prevent. Recent outbreaks of foot and mouth disease in Europe have been traced to improperly inactivated

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124/ Brown, Joan M. Genentech's Foot and Mouth Vaccine Could Find Major Markets after Third World Trials. Genetic Engineering News, Jan./Feb. 1983. p. 11.

125/ Ibid.

126/ Bittle, et al. Protection Against Foot-and-Mouth Disease by Immunization with a Chemically Synthesized Peptide Predicted from the Viral Nucleotide Sequence, p.30.

127/ Abelson, Philip H. Foot-and-Mouth Disease Vaccines. Science, v. 218, Dec. 17, 1982. p. 1181.

virus. 128/ The United States Government strictly forbids entry of both FMD viruses and vaccines to the mainland. All U.S. studies of foot and mouth disease are done on Plum Island, about a mile and a half off the coast of Long Island. 129/

An important step in solving the FMD vaccine problem has been taken by the Department of Agriculture (USDA), and Genentech, Inc., a biotechnology company in South San Francisco. Using recombinant DNA technology, Genentech researchers have succeeded in developing bacteria which produce large quantities of foot and mouth disease viral protein. The foot and mouth disease virus is surrounded by a coat containing four major proteins. Only one of them, viral protein three (or VP3), triggers the animal's immune system to produce antibodies against the FMD virus. Since VP3 alone cannot cause foot and mouth disease it was viewed as the perfect candidate for a recombinant DNA-produced vaccine.

The bacterially produced protein has been tested as an FMD vaccine at Plum Island by USDA and Genentech researchers. Two injections of the preparation, each containing 250 milligrams of VP3, were given to cattle and swine. The vaccinated animals showed a good antibody response and remained disease-free after exposure to foot and mouth virus. Field trials of the vaccine will begin in Argentina and Columbia, where FMD is endemic, in late 1983. The vaccine will be marketed by International Minerals and Chemicals (IMC), Chicago. 130/

The recombinant DNA vaccine has two major advantages over traditional preparations. First, the vaccine is safe because it does not contain a virus;

128/ Bittle, Protection Against Foot-and-Mouth Disease by Immunization with a Chemically Synthesized Peptide Predicted from the Viral Nucleotide Sequence, p. 30.

129/ Brown, Genentech's Foot and Mouth Vaccine Could Find Major Markets after Third World Trials, p. 11.

130/ Alper, Joseph. Vaccine Research Gets New Boost. High Technology, Apr. 1983. p. 62.

it merely contains noninfective viral protein. Second, the new vaccine is stable and it can withstand a temperature of 100° C, the boiling point of water. 131/ However, the USDA/Genentech vaccine was made from only one strain of foot and mouth virus. A comprehensive vaccine that protects against all 30 foot and mouth disease viral strains, or at least the major ones, awaits further research. According to one estimate, a recombinant DNA vaccine against foot and mouth disease may not be on the market before the mid-1980s. 132/

Rabies

Rabies is caused by a neurotropic virus (one with an affinity for the nervous system) that may affect all species of warm-blooded animals, including man. The frequently fatal infectious disease is transmitted almost exclusively by the bite of carnivorous animals, such as dogs, cats, raccoons, wolves, foxes, bats, and skunks. Rabies symptoms are those of profound nervous system disturbance--excitement, aggressiveness, and madness followed by paralysis and death. 133/

In 1885, Louis Pasteur developed the first rabies vaccine, using dehydrated spinal cords from rabies-infected rabbits. Today, rabies virus can be cultured in vitro (in laboratory culture systems), concentrated, and inactivated to produce a vaccine that is more potent and more easily tolerated than Pasteur's. 134/

Despite treatment advances, rabies is still a worldwide problem. In the United States, mandatory vaccination programs have nearly eradicated rabies

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131/ Abelson, Philip H. Foot-and-Mouth Disease Vaccines. Science. p. 1181.

132/ The New Vaccines. Chemical Week, Nov. 24, 1982. p. 45.

133/ Stedman's Medical Dictionary, 22d Edition, p. 1057.

134/ Yelverton, Elizabeth, et al. Rabies Virus Glycoprotein Analogs: Biosynthesis in Escherichia coli. Science, v. 219, Feb. 11, 1983. p. 614.

in dogs, but the disease is still widespread in wild animals. In South America, the death of cattle infected by rabid vampire bats costs farmers more than $28.6 million every year. 135/ 136/

The currently available rabies vaccine has many of the problems already discussed in conjunction with inactivated and attenuated preparations. Several research groups have been actively working on a virus-free rabies vaccine. The gene for a rabies antigen was first isolated from rabies virus at the Wistar Institute in Philadelphia. 137/ Scientists had previously determined that this antigen, a viral surface protein, was capable of stimulating the immune system to make antibodies against rabies.

Researchers at Transgene, a French genetic engineering company, and Genentech, a U.S. firm, have independently placed the gene for this antigen into Escherichia coli, enabling the bacteria to synthesize the rabies antigen. 138/ 139/ This bacterially synthesized rabies antigen will react, in in vitro studies, with rabbit antibodies against rabies, indicating that the antigen will be useful in eliciting the necessary immune response in whole animals. A vaccine prepared from the synthesized rabies antigen could be administered orally, by placing it in food baits, thus preventing rabies in wild animals. 140/

135/ Callas, J. J., G. C. Poppensiek and D.H. Ferris. Surveillance and Control of Virus Diseases: Americas. In Gibbs, E. P. J., ed. Virus Diseases of Animals, v. 1: International Perspectives. New York, Academic Press Inc., 1981. p. 56.

136/ Yelverton, et al. Rabies Virus Glycoprotein Analogs: Biosynthesis in Escherichia coli, Science, p. 614.

137/ Transgene Produces Surface Protein of Rabies Virus. Genetic Engineering Letter, Aug. 10, 1982. p. 3.

138/ Ibid.

139/ Chen, A. Biosynthesis of a Rabies Vaccine, Science News, v. 123, Feb. 12, 1983. p. 103.

140/ Yelverton, et al., Rabies Virus Glycoprotein Analogs: Biosynthesis in Escherichia coli, p. 614.

Scientists have cautioned that they have not yet achieved a genetically engineered rabies vaccine. More tests of further purified viral antigens are needed. A commercial vaccine may not be available until 1986.

Parvovirus

Parvovirus infects a variety of hosts including mink, cats, dogs, cattle, swine and humans. Disease symptoms can include fever, dehydration, inactivity, diarrhea, and vomiting. An acute epidemic of canine parvovirus occurred in the United States during the summer of 1978, and a shortage of vaccines resulted. 141/ Although the shortage of canine vaccines has been alleviated, a safer more effective product is still required and could possibly be developed through genetic engineering.

Porcine parvovirus is an important cause of reproductive loss in swine. The infected sow remains healthy but transplacental infection of pig fetuses during the first half of gestation can lead to embryonic death and resorption or mumification, with few surviving normal fetuses. 142/ Bovine parvovirus appears to be an important contributor to calf losses from neonatal diarrhea and abortion. The virus has been recovered from feces of normal calves and calves with diarrhea, as well as from aborted fetuses. 143/ 144/ Vaccines have not been developed for

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141/ Siegmund, Otto H. and Clarence M. Fraser, eds. The Merck Veterinary Manual: A Handbook of Diagnosis and Therapy for the Veterinarian, 5th ed. Rahway, N.J., Merck and Co., Inc., 1979. p. 305-306.

142/ Derbyshire, J. B. Virus Diseases of Pigs. In Gibbs, E. P. J., ed. Virus Diseases of Food Animals, v. 1: International Perspectives. New York, Academic Press Inc., 1981. p. 185-186.

143/ Kahrs, R. F. Virus Diseases of Cattle. In Gibbs, E. P. J., ed. Virus Diseases of Food Animals, v. 1: International Perspectives. New York, Academic Press Inc., 1981. p. 148.

144/ Kahrs, R. F. Viral Diseases of Cattle. Ames, IA, The Iowa State University Press, 1981. p. 183.

porcine or bovine parvovirus at the present time. However, two genetic engineering companies (Applied Molecular Genetics, or Amgen, located in Thousand Oaks, CA and Molecular Genetics in Minnetonka, Minnesota) are working on a porcine parvovirus vaccine. 145/

Bovine Viral Diarrhea

Bovine Viral Diarrhea, BVD (also known as Mucosal-disease complex), is an infectious disease of cattle characterized by ulcerations of the mouth, pharynx, esophagus, stomach and intestines. The infection is usually accompanied by fever and severe diarrhea, and is also associated with congenital arthritis, abortion and defects of the brain in young calves. 146/ Acute, chronic and subclinical BVD can occur, with the acute cases usually being fatal. 147/ Subclinical BVD can predispose the animal to other infections. Because cattle with the acute systemic form usually die, and serious chronic cases are usually culled, it is the subclinical infections that are followed by abortion or the birth of calves with congenital anomalies which cause the major economic impact of BVD. 148/

Both inactivated (killed) and attenuated (modified-live) BVD vaccines are currently available. 149/ The attenuated virus vaccine is not approved for use

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145/ Genetic Technology News, v. 3, no. 6, June 1983, p. 3.

146/ Siegmund and Fraser, The Merck Veterinary Manual, p. 266.

147/ Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 90-91.

148/ Ibid., p. 91.

149/ U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. Veterinary Biological Products: Licensees and Permittees. Issued Jan. 1983. p. 13-14.

in pregnant cattle because it can induce abortion. This vaccine has also been associated with post-vaccination mucosal disease. 150/ Genetic engineering companies have begun work on a recombinant DNA vaccine for BVD.

Papillomavirus

Papillomavirus infection produces warts in a variety of animals including rabbits, cows, pigs, dogs, horses, goats and humans. The warts commonly occur on the head, neck and shoulders, and occasionally on the back and abdomen. The veneral form of the wart can be a serious problem in young bulls and can cause difficulties in calving for heifers when it affects the vaginal mucosa. 151/ "Economic loss occasionally results when lesions become generalized and excessive, penile warts interfere with breeding, or warts on teats interfere with milking or predispose to mastitis" 152/ The presence of warts can cause the reduction of hide value, and the rejection of cattle for export or for participation in shows. Warts occur most commonly in young cattle and usually disappear within one year. Commercial vaccines provide partial protection if administered repeatedly prior to infection. 153/ Molecular Genetics (Minnetonka, Minnesota) is developing a bovine papilloma virus vaccine using recombinant DNA technology. 154/

150/ Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 101-102.

151/ Siegmund and Fraser, Merck Veterinary Manual, p. 251.

152/ Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 121.

153/ Ibid.

154/ Genetic Technology News, June 1982, p. 4-5.

Rift Valley Fever

Rift Valley fever (RVF) is a mosquito-borne viral disease that affects sheep, goats, camels, cattle, buffaloes, antelopes, rodents and humans. RVF is characterized by a short incubation period, fever, hepatitis, death in young animals and abortion in pregnant animals. 155/ The disease is confined to Africa but has the potential to spread to other areas in the world. It is named for the Rift Valley in Kenya, where it was first discovered. Mortality rates approach 90 percent in lambs. Adult sheep and cattle are less seriously affected but calves may experience high death rates. 156/ Outbreaks of RVF are usually sudden and spread rapidly during the rainy season when mosquitos are active. Current control methods include spraying or using other mosquito control procedures and vaccinating animals with live virus preparations which must be administered annually. Pregnant animals are vaccinated with killed virus preparations. Genetic engineering companies are attempting to develop a vaccine against Rift Valley fever.

Bluetongue

Bluetongue (BT) is an insect-transmitted viral disease affecting sheep, cattle, goats and wild ruminants. 157/ BT is principally a disease of sheep,

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155/ Siegmund and Fraser, Merck Veterinary Manual, p. 437.

156/ Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 270.

157/ Siegmund and Fraser, Merck Veterinary Manual, p. 251.

but cattle can also become infected and are thought to be carriers of the disease. The BT virus is transmitted by gnats and is not spread by direct contact between animals. The name bluetongue is derived from the blue discoloration of the tongue which is sometimes observed in sheep but rarely seen in cattle. BT infections in sheep are characterized by fever, nasal discharge, mouth sores, swollen blue tongue and lameness. In cattle, BT infections are usually subclinical with inapparent symptoms, but a rare, fatal, usually sporadic disease has been associated with BT infections in cattle. 158/

Because cattle are thought to be carriers for the disease in sheep, some sheep-producing nations prohibit the importation of cattle or bovine semen from BT-infected areas. 159/ 160/ The virus which causes BT is present in many Southern and Western States. 161/ The disease costs the U.S. livestock industry millions of dollars annually due to loss of sales of cattle, semen and embryos to overseas markets. 162/ Control of BT is difficult because of its complex epidemiology. Vaccines are not currently available for cattle. Attenuated virus vaccines are available for use in sheep; killed virus vaccines are being developed. Researchers at the USDA Plum Island Animal Disease Center are developing a recombinant DNA BT vaccine. A viral coat protein

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158/ Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 71.

159/ Ibid.

160/ Genetic Technology News, v. 3, Feb. 1983, p. 6.

161/ Genetic Engineering Letter, v. 3, Jan. 10, 1983, p. 2.

162/ Genetic Technology News, v. 3, Feb. 1983, p. 6.

of one BT strain has been identified and efforts are now underway to clone its corresponding gene. 163/ 164/

Transmissible Gastroenteritis

Transmissible Gastroenteritis (TGE) is a viral disease of swine characterized by profuse diarrhea and vomiting. TGE rapidly spreads through swine herds causing very high mortality in piglets under two weeks of age due to dehydration. In older animals, the disease is less severe with much lower mortality, but lactating animals may be severely affected. 165/ Outbreaks of TGE usually occur in winter and early spring. The disease is thought to be transmitted by infected swine which shed the virus for up to eight weeks after infection. It may also be spread through contact with infected cats, dogs, foxes, or starlings. 166/ The virus is readily transmitted through feces. TGE may also be contracted via airborne virus if the animals are kept in confined areas. The virus can be destroyed by sunlight and heat but may remain infective for years when frozen. 167/

There is no specific treatment for TGE infections. Modified live virus vaccines for the control of the disease have been of limited value. 168/

163/ Ibid.

164/ Genetic Engineering Letter, v. 3, Jan. 10, 1983, p. 2.

165/ Sigmund and Fraser, Merck Veterinary Manual, p. 293.

166/ Derbyshire, Virus Diseases of Pigs, p. 189.

167/ Sigmund and Fraser, Merck Veterinary Manual, p. 293.

168/ Derbyshire, Virus Diseases of Pigs, p. 189.

Applied Molecular Genetics (Amgen) is in the process of developing a TGE vaccine using recombinant DNA techniques. 169/

Bacterial Vaccines

Colibacillosis

Colibacillosis, or "scours," is caused by a pathogenic strain of Escherichia coli which colonizes the intestines of piglets and calves shortly after birth. The bacterium attaches itself to the animal's intestinal wall with tiny projections, called pili, and then produces a lethal toxin. 170/ The disease causes extensive losses in newborn calves and pigs, killing about 10 percent of the animals. 171/ Conventional vaccines are available but they are not very effective or long lasting and tend to vary in quality.

The very first products to be made using genetic engineering and marketed were vaccines for porcine and bovine scours developed by Intervet, a subsidiary of Akzo, the Dutch chemical and textile company. 172/ Intervet's vaccine is composed of the protein which forms the pili of the pathogenic bacteria. The protein, called K88 in the case of pigs and K99 for cattle, is manufactured by genetically engineered bacteria. When the vaccine is injected into a pig or cow, the animal will make antibodies against the pili protein which prevent the attachment of pathogenic E. coli. Because the

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169/ Amgen 1983 Company Prospectus, p. 16.

170/ First Genetically Engineered Product on Sale. New Scientist, May 6, 1982. p. 336.

171/ Ibid.

172/ Ibid.

infection occurs very rapidly, the vaccine is given to pregnant sows or cattle, who pass the antibodies to their nursing young via their milk.

Cetus Corporation (Berkeley, California) has developed a porcine scours vaccine using the same approach as Intervet, described above. The vaccine will be marketed by Norden Laboratories (Lincoln, Nebraska) which is a subsidiary of SmithKline Beckman (Philadelphia, Pennsylvania). 173/ Cetus Corp. is also working with TechAmerica (Elwood, Kansas) to develop a scours vaccine for calves. 174/ Salsbury Laboratories, a U.S. subsidiary of Belgium's Solvay Group, is marketing a porcine scours vaccine called EcoBac, developed by Richard E. Isaacson of the University of Michigan and its newly established Center for Molecular Genetics. 175/ 176/ The vaccine has been approved by USDA, and Salsbury Laboratories has started manufacturing the vaccine at its Charles City, Iowa plant. 177/

In contrast to the vaccines discussed above, an oral monoclonal antibody has been developed by Molecular Genetics, Inc. (Minnetonka, Minnesota) to prevent scours in newborn calves. 178/ The antibodies are given to the animals in their routine feed supplements and are made using cell hybridization (hybridoma) techniques, as previously discussed. Since the calf begins producing its own antibodies three days after birth, the animal is susceptible to

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173/ Dwyer, Paula E. Fildes Shifts Cetus Policies as New Vaccine is Launched. Bio/Technology, v. 1, June 1983. p. 313-314.

174/ Genetic Technology News, v. 3, Feb. 1983, p. 4.

175/ Ibid., p. 3-4.

176/ Genetic Engineering News, v. 3, Mar./Apr. 1983, p. 4.

177/ Genetic Technology News, v. 3, Feb. 1983, p. 3.

178/ Genetic Technology News, v. 2, Aug. 1982, p. 7.

scours only during the first two days of life. 179/ The product developed by Molecular Genetics, Inc. lasts somewhat longer than two days and therefore is able to keep the animal from developing the disease. Following clinical trials, the product was approved for sale in Canada and will be marketed there by Vetrepharm (London, Ontario, Canada). 180/

Mastitis

Mastitis, or inflammation of the mammary gland, is a complex disease caused by a variety of infectious agents, and therefore is difficult to treat. According to information returned in the October 1981 survey of dairy farmers conducted by the National Dairy Herd Improvement Association, 95 percent of dairy farmers treat 25 percent of their milking herd for mastitis annually and spend over $500.00 per farm for mastitis treatments alone. This figure does not include the cost of veterinary service, mastitis screening, loss of income from reduced milk production and culling due to mastitis. It is estimated that in 1981, the dairy industry lost over $1 billion because of mastitis-related decreases in milk production and discarded milk.

At the present time, mastitis is treated by keeping the animal clean and by using antibiotic and anti-inflammatory drugs. Researchers are developing preparations of monoclonal antibodies to induce passive immunity against the pathogens found most often in association with mastitis. Monoclonal antibodies could also be used in the diagnosis of this disease, resulting in early treatment and decreased milk loss. Such immunotherapeutic agents would probably not be available before 1985.

179/ Alper, Vaccine Research Gets New Boost, p. 62.

180/ Genetic Technology News, v. 3, Apr. 1983, p. 3.

Protozoal/Parasitic Vaccines

A parasite is an organism that lives on or in another species, obtaining food and shelter at the host's expense. Normally, parasites cannot exist without a host. Parasitic animals include many protozoans, such as Plasmodium which causes malaria, flukes, tapeworms and other flatworms, and many roundworms. Protozoal parasites are an important cause of disease and death in humans and domestic animals. Millions of acres of land in Africa cannot be used for cattle grazing because of infestation with tick-borne diseases, such as trypanosomiasis. Tropical and subtropical hemoparasites, which infect the blood, helminthic parasites (worms) and intestinal protozoa, such as coccidia, cause extensive losses in livestock and poultry.

A major problem in developing immunotherapeutic agents to prevent parasitic diseases is the complexity of the parasitic organism's life cycle. Each stage of a parasite's life cycle may be passed in a different host or carrier, making the organism difficult to study in laboratory cultures. Furthermore, many parasites go through extensive antigen changes between each life cycle stage. Trypanosomes, for example, have at least 104 different antigens. While researchers have begun work on a recombinant DNA malaria vaccine, more basic research on the biology of parasites is needed before practical vaccines can be produced for some of the other parasitic diseases discussed below.

Coccidiosis

Coccidiosis is a parasitic disease caused by one or more species of host-specific protozoans, called coccidia, which infest the host's digestive tract and destroy its inner lining. The disease is relatively common in many animals, and is a serious disease problem in cattle, sheep, goats,

chickens and turkeys. 181/ Infection of the host animal results from ingestion of infective oocysts, the encapsulated intermediate stage in the life cycle of some parasites. Oocysts enter the hosts environment when they are discharged in the feces of an infected animal. 182/ Following ingestion, the infective organisms are released from the oocyst and invade the inner lining of the host's intestines.

Vaccines for the prevention of coccidiosis have not yet been developed. Clinical infections are self-limiting and subside within a week if continuous reinfection does not occur. Medication slows or inhibits reinfection and therefore may shorten the course of infection, reducing the discharge of oocysts, alleviating hemorrhage and diarrhea, and decreasing the chance of secondary infections and mortality. 183/

Trypanosomiasis

Sleeping sickness, or trypanosomiasis, is caused by parasitic protozoans, called trypanosomes, which infect the blood of man and many domestic animals. Trypanosomes usually pass through an intermediate host, such as a leech or tick, before infecting vertebrates. 184/ The African cattle industry loses $5 billion annually because large areas of land are infected with trypanosomiasis and cannot be used for cattle grazing. 185/ Since this protozoan has evolved a complex set of antigens which appear sequentially during its lifecycle, attempts

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181/ Siegmund and Fraser, Merck Veterinary Manual, p. 458.

182/ Ibid.

183/ Ibid., p. 461.

184/ Ibid., p. 420.

185/ Alper, Joseph. Vaccine Research Gets New Boost. High Technology, Apr. 1983. p. 62.

to use trypanosome antigens as a basis for a genetically engineered vaccine may prove to be difficult. Genetic engineering techniques will be useful in basic research studies designed to learn more about gene regulation in these parasites. Until scientists discover which antigens are significant and determine the types of response needed to impart immunity, recombinant DNA technology cannot be applied directly to the disease itself.

Malaria

Organisms belonging to the genus Plasmodium have a worldwide distribution in reptiles, birds and mammals; 22 species have been described as affecting primates alone. 186/ There are an estimated 150 million new human cases of malaria per year. 187/ The World Health Organization estimates that 107 countries are affected and that about half the world's population is exposed to the risk of infection. 188/ The disease has a very high mortality rate in children; approximately one million children in Africa die from malaria each year. 189/

Malaria is transmitted by the bite of infected Anopheles mosquitoes. Anti-malarial drugs are available, but the emergence of drug resistant

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186/ Warren, McWilson. Malaria. In Hulbert, William, William F. McCulloch and Paul R. Schnurrenberger, eds. Disease Transmitted From Animals to Man, 6th edition. Springfield, Ill., Charles C. Thomas, Publisher, 1975. p. 789.

187/ Davidson, George. Who Doesn't Want To Eradicate Malaria? New Scientist, Dec. 16, 1982. p. 732.

188/ Ibid.

189/ Priorities in Biotechnology; Research for International Development. Proceedings of a Workshop, July 26-30, 1982. Washington, D.C., National Academy Press, 1982. p. 79.

Plasmodium in Southeast Asia and South America pose a major health threat. Control of the disease is also being hampered by insecticide-resistant mosquitoes. Vaccines against malaria are not currently available. Scientists at New York University are working on a recombinant DNA-derived malaria vaccine. The research is being funded by the World Health Organization and the U.S. Agency for International Development. 190/

Babesioses

Babesioses, which resembles human malaria, is caused by tick-borne parasites of the genus Babesia which infect and develop within the red blood cells of mammalian hosts. It is a significant disease problem in domestic and wild animals wherever tick-vectors are present, but is a particular problem in the tropics. 191/ Babesioses has also been reported as occurring in man. 192/ The disease is characterized by malaise, fever and anemia. When the central nervous system becomes infected, mania and loss of muscle coordination, followed by coma or death can result. 193/

In endemic areas, animals often become exposed to the parasite in the first year of life. It is thought that they are protected during the first year by maternal antibodies transferred via milk (for about two months), or

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190/ Rights Row Clouds Malaria Breakthrough. New Scientist, Dec. 23-30, 1982. p. 781.

191/ Siegmund and Fraser, Merck Veterinary Manual, p. 426.

192/ Wescott, Richard B. Piroplasmosis. In Hubbert, William T., William F. McCulloch and Paul R. Schnurrenberger, eds. Disease Transmitted From Animals to Man, 6th edition. Springfield, Ill., Charles C. Thomas, Publisher, 1975. p. 799.

193/ Seigmund and Fraser, Merck Veterinary Manual, p. 429.

by a temporarily elevated innate resistance. One exposure to Babesia parasites during this period protects most animals from subsequent clinical attacks. Losses to livestock herds (cattle, sheep, goats) occur when: animals escape exposure during the first year, and therefore are not immune; when susceptible animals are moved to endemic areas; or when the parasite-infected ticks move to previously uninfested areas. 194/

Live virus vaccines are used in Australia and parts of South America to control the disease. 195/ Babesiosis has been eliminated in the United States through tick eradication programs, however, resistant ticks now limit the usefulness of this method. 196/ International Minerals and Chemicals, a Chicago company, is sponsoring research at the University of Illinois on vaccines effective against bovine and canine babesiosis. 197/ Dr. Miordag Ristic, a veterinary scientist at Illinois, found that when Babesia enters a red blood cell, it sheds substances called glycoproteins which are essential for the infection process. Glycoproteins are proteins which have attached carbohydrate (sugar) groups. By themselves, the glycoproteins cannot elicit an immune response, but when combined with the carrier saponin, a plant extract, they make very good vaccines. The bovine vaccine has passed preliminary tests, and field trials in Venezuela and Australia are already under way. 198/

194/ Seigmund and Fraser, Merck Veterinary Manual, p. 426-427.

195/ Ibid.

196/ Alper, Vaccine Research Gets New Boost, p. 62.

197/ Ibid.

198/ Ibid.

B. INTERFERONS

Cells in culture and in live animals produce substances called interferons in response to infection by live or inactivated virus. Interferons are small proteins which induce a state of resistance against an invading virus. They are also able to "interfere" with viruses other than the one that evoked their production, but are usually only effective on cells of the species which have produced the interferon. 199/ It is thought that these natural body products might be useful in treating cancer and certain viral infections, such as colds.

Research on interferon has been handicapped by the difficulty and expense of extracting and purifying large quantities of the substance. A number of genetic engineering firms, however, have succeeded in cloning and producing several of the many different types of human interferon. Researchers at Genentech (South San Francisco, California) have produced bovine interferon by a recombinant DNA process. The bovine interferon will be tested for its effectiveness in treating viral diseases in cattle. 200/ 201/

Recently, Genentech representatives met with officials of the U.S. Department of Agriculture and the Food and Drug Administration to determine whether their bovine interferon should be regulated as an animal drug by the FDA or as an animal biologic by the USDA. This jurisdictional question must be answered before product regulation or further testing may continue. 202/

199/ Stedman's Medical Dictionary, 22d ed., p. 640.

200/ Genetic Technology News, Apr. 1982., p. 4.

201/ The Livestock Industry's Genetic Revolution. Business Week, June 21, 1982. p. 130.

202/ Jurisdiction Clarification Sought on Recombinant DNA Bovine Interferon. Food Chemical News, Apr. 4, 1983. p. 20.

C. DIAGNOSTICS/MONOCLONAL ANTIBODIES

As discussed in a previous section, monoclonal antibodies, are produced by a clone of cells derived from a single hybridoma cell. Hybridomas are formed by the fusion of a malignant cell (called myeloma) with an antibody-producing cell. Monoclonal antibodies can be used in diagnostic tests to detect the presence of an infection or cancer in blood or body tissue samples. To facilitate this procedure, the monoclonal antibodies are usually tagged with radioactive labels that allow them to show up under certain laboratory conditions. Monoclonal antibodies have been prepared against a number of human pathogenic viruses, bacteria and parasites. 203/ Monoclonal antibody-based diagnostic tests are also being developed and used for diseases which affect livestock. Their use will probably increase as techniques are improved, new antibodies developed and costs lowered.

D. ANIMAL HORMONES

Bacteria can also be used to manufacture animal hormones, natural substances that can speed growth, enhance milk production, and increase the efficiency of feed use. The necessary gene (or genes) must be isolated, inserted into a virus or plasmid, and then into bacteria. As the bacteria reproduce, they replicate the inserted gene along with their own genetic material and then use the instructions provided by the gene to manufacture the desired hormone.

203/ Nowinski, Robert C., et al. Monoclonal Antibodies for Diagnosis of Infectious Diseases in Humans. Science, v. 219, Feb. 11, 1983. p. 638.

Growth Hormone

Feed additives and drugs that promote animal growth represent a multimillion dollar business. However, feed additives have developed a bad reputation among consumers. DES, for example, has been used as a feed additive and/or subcutaneous animal implant. Other uses of DES have been associated with the development of human cancer. An alternative that may soon be available is growth hormone, a pituitary product that naturally promotes growth.

In the past, using growth hormone has been very expensive, because the hormone had to be extracted from animal pituitary glands. To obtain one gram of bovine growth hormone, for example, required the glands from one thousand cows. 204/ However, genetically engineered bacteria can now be used to cheaply and quickly produce large quantities of growth hormone. Such a possibility is already being realized for chicken growth hormone. Applied Molecular Genetics, Inc. (Amgen), a biotechnology firm based in Thousand Oaks, California has succeeded in splicing the chicken growth hormone gene into bacteria. The genetically engineered bacteria have produced enough hormone for testing purposes. 205/

Initial tests of the recombinant DNA growth hormone indicate that it can increase a chicken's growth rate by 15 percent. If further tests prove efficacy, and if the FDA approves the hormone for use as an additive in chicken feeds, the product may be on the market by the end of 1984. 206/ Scientists at Amgen have explained that the growth hormone was developed not to make bigger chickens, but to grow normal sized chickens faster. By sending the

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204/ The Livestock Industry's Genetic Revolution. Business Week, June 21, 1982. p. 130.

205/ Biotechnology Struts into the Hen House. Business Week, Apr. 11, 1983. p. 36.

206/ Ibid.

birds to market sooner, farmers should be able to save expenses on feed costs. 207/

Monsanto Corp. and Genentech are collaborating on the development of bovine and porcine growth hormone produced by genetically engineered E. coli. Tests are underway to assess the safety and efficacy of these products and if successful, they should be on the market by 1990. 208/ 209/ A study at Cornell University indicated that bovine growth hormone could increase milk production in cows by as much as 40 percent with no increase in feed consumption. 210/ More conservative estimates place the increase at around 15 to 17 percent. 211/ Other companies working on bovine growth hormone include Molecular Genetics, Inc., collaborating with American Cyanamid (Wayne, New Jersey) and Collaborative Genetics (Lexington, Massachusetts) with AKZO Pharma (Netherlands). 212/

Prolactin

Prolactin is the pituitary hormone that stimulates the secretion of milk. It may also stimulate breast growth during pregnancy. 213/ Much less is known about the role of prolactin than about the importance of growth hormone in

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207/ Orr, Richard. Superchicken: Cloning May Yield Superior Poultry. Chicago Tribune, Apr. 17, 1983. p. 4:6.

208/ Genetic Technology News, Jan. 1982, p. 3.

209/ Genetic Technology News, Aug. 1982, p. 1.

210/ The Livestock Industry's Genetic Revolution. Business Week, p. 130.

211/ Gorewit, R. C. et al. Improved Animal Efficiency Using Pituitary Hormones. San Francisco, Conference on Biotechnology: Commercial and Industrial Applications and Opportunities, 1982. p. 112-113.

212/ Genetic Technology News, Aug. 1982, p. 1.

213/ Stedman's Medical Dictionary, 22d ed., p. 1023.

biological processes. Prolactin may be capable of altering metabolism, directly or indirectly, in several tissues, such as muscle and fat. The result is enhanced growth of lean body tissue. Some studies have linked increases in prolactin with enhanced growth and milk production. 214/ As in the case of growth hormone, described above, it is possible to use recombinant DNA technology to synthesize prolactin. Both growth hormone and prolactin could be used to improve the efficiency of meat and milk production.

Hormone Delivery and Slow-Release Technology

An effective method of administering hormones at regular intervals must be developed before they can be used commercially. A study at Cornell University compared three different forms of bovine growth hormone administration in Holstein cows. The hormone was delivered in one of the following forms: daily subcutaneous injections; continuous intravenous infusion throughout the day; or pulsed intravenous infusion six times a day. The study revealed that all three forms of administration were effective in increasing milk yield. According to these results, it is the amount of growth hormone, not the way in which it is delivered, that is important. 215/

Since daily injections and intravenous infusions of livestock would be time consuming and expensive for commercial use, more practical methods of hormone delivery are being developed which will be convenient for the farmer and still effective in enhancing growth and milk production. These methods allow the hormone to be released slowly over a fairly long period of time.

214/ Gorewit, et al., Improved Animal Efficiency Using Pituitary Hormones. p. 115-116.

215/ Ibid., p. 114-115.

A slow-releasing ear implant is currently being used, and a long-lasting bolus (a very large, soft pill) that slowly releases the hormone in the stomach has been developed.

E. ANIMAL NUTRITION

The ultimate goal of studies on the nutritional needs of agricultural animals is to increase productivity while decreasing feed requirements, thereby enhancing the efficiency with which human food needs are met. Progress being made in improving the nutrient quality of feed grains, developing nutritious additives for animal feed, and altering the intestinal microorganisms of animals (to allow livestock to derive sustenance from ordinarily indigestible matter) is taking science closer to this goal.

Feed Grain Improvement

Perhaps one of the most effective ways of using the new biotechnology for improving animal nutrition will be the improvement of food plants through genetic engineering. Modern crop management, while it has been able to increase plant quality and productivity, has its drawbacks. For example, current management regimens typically use increasing amounts of expensive nitrogen fertilizers, and must often resort to the use of pesticides and herbicides, which can leave toxic residues in the environment. Furthermore, modern plant breeding has decreased the pool of available genes. Without sufficient potential for genetic variation, some plant crops could be completely destroyed should a major disease outbreak occur. It is also possible that modern inbreeding could result in the loss of important genetic traits. 216/

216/ Barton, Kenneth and W. J. Brill. Prospects in Plant Genetic Engineering. Science, v. 219, Feb. 11, 1983. p. 671.

Recombinant DNA technology could provide solutions to some of these problems. Conceivably, it could be used to improve the nutritional quality of feed plants, decrease nitrogen fertilization requirements, and increase plant resistance to environmental stresses (for example, drought and flood) and diseases.

However, less is known about plant genetics on the molecular level than, for example, about viruses and bacteria, and there are fundamental obstacles that must be overcome if genetic engineering is to be successful in plants.

> Plant cells are eucaryotic; their cells contain nuclei in which their major store of genetic information is contained. Bacterial cells, generally considered more primitive, are procaryotes; their genetic material, contained in no nuclei, floats freely in their cytoplasm. Further or perhaps consequentially, plants just don't come accompanied by the variety of genetic entities that, as vehicles for gene transport, have made genetic engineering possible on the bacterial level. Preliminary research also indicates that regulation of plant genes may be much more complex than that of bacterial genes. Researchers have recently found that the plant genes considered most desirable to transfer are likely to be part of multigene systems that will be difficult to manipulate. It is not yet clear that just time, money, and the translation of genetic-engineering information and technology from zoology to botany will solve these problems. 217/

Despite these obstacles, many scientists believe that genetic manipulation of plants is possible and even essential given our increasing need for human and animal food. 218/

Current plant genetics research is following several lines, including protoplast formation, cell fusion and regeneration, the development of new plant strains from mutant cells, and the identification of vectors to carry foreign genes into the plant cell nucleus. Cell fusion studies use protoplasts, which are formed by using enzymes to remove the cell walls from plant cells. Protoplasts of two different plant species are then fused, forming

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217/ Check, William A. Engineering the Botanical Gene. Mosaic, v. 13, May/June, 1982. p. 19.

218/ DeYoung, H. Garrett. Crop Genetics: The Seeds of Revolution. High Technology, June, 1983. p. 53.

a hybrid which contains the genetic material of both original cells, a mixture that could not take place naturally. Scientists then attempt to generate a whole plant from the hybrid cells. 219/ Only a few such fusions have actually succeeded. For example, tomato cells have been fused with potato cells, but the regenerated hybrid plant was sterile, and thus incapable of producing seeds. 220/

Scientists are also trying to create new crops by culturing leaf cell protoplasts which seem to be capable of asexually generating genetic variants. Some of these genetic variants may be useful to plant breeders. It is not known whether the genetic changes which appear are the result of genetic differences in the leaf cells or of the process of forming and culturing the protoplast cells. The cells are allowed to proliferate and regenerate into whole plants that are tested for survival traits and inheritability of their new characteristics. 221/

Early this year, scientists at Monsanto reported the successful implantation of a bacterial gene into plant cells. The Monsanto team placed the bacterial gene for resistance to the antibiotic Kanamycin into petunia, tobacco, sunflower, and carrot seeds. This was accomplished by using Agrobacterium tumefaciens, a bacteria which causes crown gall disease (a type of tumor) in many varieties of plants. A. tumefaciens contains a plasmid (a circular piece of DNA) which is responsible for the plant tumor producing properties of the bacteria. In the crown gall disease process, the plasmid is released from the bacteria, enters a plant cell and becomes integrated into the plant's DNA. The scientists at Monsanto made use of this property by placing the gene for Kanamycin resistance

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219/ Solomon, Stephen. Plants that Break the Gene Barrier. Science Digest, Apr., 1983. p. 18.

220/ Shepard, James F. The Regeneration of Potato Plants from Leaf-Cell Protoplasts. Scientific American, May 1982. p. 166.

221/ Ibid., p. 164.

into the A. tumefaciens plasmid. The plasmid was then inserted into the A. tumefaciens bacteria, and the bacteria was allowed to be mixed with normal plant cells. The incorporation of the Kanamycin-restance gene from the plasmid into the plant cells was demonstrated by the fact that some of the plant cells were able to survive and grow on media containing Kanamycin, which would ordinarily kill normal plant cells. This work represents the first time an engineered gene has been transferred and expressed in a plant. 222/ Scientists hope to use A. tumefacines and its plasmid in future studies to transfer agriculturally useful genes which would provide traits such as:

disease and pest resistance;
higher yield;
accelerated growth rates;
temperature tolerance;
crops developed for arid regions;
tolerance to high salt concentrations;
increased photosynthesis efficiency;
improved amino acid balance in grain proteins; and
nitrogen fixing ability.

Feed Additive Production- Amino Acids

Proteins are large, complex molecules consisting of long chains of amino acids. Plants are capable of synthesizing all twenty amino acids required for protein production from simple sugars and nitrogen-containing precursor molecules. Most animals including humans, are unable to synthesize ten of the amino acids which must, therefore, be supplied in the diet. These ten amino acids are usually referred to as the "essential amino acids." Finding better ways of supplying these essential amino acids to agricultural animals is the focus of many research projects.

222/ DeYoung, Crop Genetics, p. 54-55.

Plants store protein, made of amino acids, in their seeds which are often used as animal feed. However, these storage proteins don't always contain all ten essential amino acids. The plant does not need to have a ready supply of all the amino acids, since it can manufacture them for itself. One solution, as mentioned in the list on the previous page, would be to use genetic engineering techniques to improve the amino acid balance of grain proteins. This would be accomplished by inserting the gene for a desired storage protein into, for example, corn plant cells. The much publicized "sunbean" is one such attempt in which a protein gene from a bean plant was inserted into sunflower tissue culture cells. 223/ The scientists, however, were unable to induce the mass of "sunbean" cells to form a viable plant.

At the present time, amino acids are added to some livestock feed mixtures. Bacteria are used in fermentation processes to produce essential amino acids such as lysine and tryptophan, which are then added to animal feed. Recombinant DNA technology is currently being used to improve these amino acid fermentations and thus decrease the cost of these feed additives. If this possibility is realized, it could save farmers billions of pounds of feed protein annually. 224/

223/ Bean Gene Moved to Sunflower Cell. Agricultural Research, Aug. 1981. p. 4-5.

224/ Pond, Wilson G. Modern Pork Production. Scientific American, v. 248, May, 1983. p. 102.

Single Cell Protein

An alternative to feed grains, improved or otherwise, is "non-crop" protein provided by single-celled organisms. Single cell protein is the name given to protein materials derived from the controlled fermentation of substates by microorganisms. 225/ Microorganisms have been used for centuries in processes for making cheese and yogurt. Genetic engineering may allow us to use microorganisms on a much larger scale to convert non-food items (for example, sewage, methane, and woodpulp) to palatable, high quality food. The requirements are a cheaper substrate, microorganisms (fungi, algae, yeast, or bacteria), and a mechanical system to mix and aerate the fermenting mass. 226/

One such product, called "Pruteen" is composed of the bacterium Methylophilus methylotrophus. In the Pruteen production process M. methylotrophus is grown in large fermenters using methanol, ammonia and air as raw materials. Other inputs include steam, electricity, gas, phosphoric acid, sulphuric acid and various inorganic salts. Within 36 hours the bacteria have multiplied so that the fermenter contains 40 metric tons of cells. The product can then be dried into pellets which meet the size specifications required by the feed industry. The product Pruteen, like any other dietary protein material, is not a single component but a mixture of proteins, fats, minerals and trace ingredients. The protein level in Pruteen is 73 percent. Tests of Pruteen indicate that it is a safe raw material for use in animal feed. 227/

225/ Waterworth, D. G. Single Cell Protein. Outlook on Agriculture, v. 10, 1981. p. 405.

226/ Ibid., p. 405.

227/ Ibid., p. 406.

Single cell protein has several advantages over the dietary protein provided in conventional feed grain: supply is uninterrupted by bad weather; the product is consistent; and there are no contaminants. 208/

Manipulation of Intestinal Microflora

It has been estimated that animal feed grains will become less available in the 21st Century than they are at the present time in the United States. 229/ To sustain the current levels of animal production, alternative feed sources will have to be developed. One such source may be cellulose, which is a complex polysaccharide forming the basis of plant fiber. Cellulose is the most abundant organic compound in the world. 230/ Plants containing cellulose grow almost everywhere on earth, and contain as much energy as starchy crops. However, cellulose is not efficiently utilized by animals.

One possible solution would be to change the activity of intestinal microflora (bacteria) of certain agricultural animals. Recombinant DNA technology could be used to alter these natural gut bacteria so that they would make cellulase (the enzyme which cleaves cellulose) thus allowing cellulose to be digested. 231/

228/ Ibid., p. 408.

229/ Wedin, W. F., et al. Feed Production. In Animal Agriculture: Research to Meet Human Needs in the 21st Century. p. 165.

230/ Stedman's Medical Dictionary, 22d ed., p. 249.

231/ Pond, Modern Pork Production, p. 101-102.

CONCLUSION

Breeding livestock for increased productivity is an extremely slow process, especially with species such as cattle which have long generation periods. Most cattle breeders will only be able to work with six or seven generations of cattle. 232/ Nevertheless, techniques such as artificial insemination have made a tremendous impact upon, for example, increased milk production in the dairy industry. It is anticipated that the advances in reproductive and health technologies discussed in this paper will accelerate the process of selective breeding for improved traits in the near future.

A majority of these advances in animal sciences are based on discoveries made over the past 10 years in genetics and biomedical research. These discoveries reflect the commitment of serious public support for research in the natural sciences, especially during the last 30 years. Since 1950 approximately $75 billion from private and public funds have been expended for research and development on health care in the United States, and the annual investment, currently (1981) represents about 0.31 percent of the gross national product. About 60 percent of total funds and an estimated 90 percent of the basic research has been supported by the Federal budget. 233/ Although these sums are identified as health care research, it should be noted that many of the new technologies and much of the basic biology of mammalian reproductive systems have evolved

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232/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 351.

233/ Brackett, Benjamin G., George F. Seidel, Jr., and Sarah M. Seidel. Continuation and Implementation of Research. In their: New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 257.